ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2013 December 24; 3(4): 48-133

Baishideng Publishing Group
www.wjgnet.com

WJT World Journal of Transplantation

A peer-reviewed, online, open-access journal of transplantation

Editorial Board

2011-2015

The *World Journal of Transplantation* Editorial Board consists of 387 members, representing a team of worldwide experts in transplantation. They are from 43 countries, including Argentina (2), Australia (7), Austria (3), Belgium (4), Brazil (8), Bulgaria (1), Canada (15), China (32), Cuba (1), Czech Republic (1), Denmark (1), Finland (1), France (6), Georgia (1), Germany (14), Greece (6), Hungary (2), India (8), Iran (7), Israel (4), Italy (36), Japan (19), Jordan (1), Macedonia (1), Mexico (2), Morocco (1), Netherlands (5), Nigeria (1), Norway (1), Pakistan (1), Poland (2), Qatar (1), Saudi Arabia (4), Singapore (1), South Korea (17), Spain (9), Sweden (1), Switzerland (3), Thailand (2), Tunisia (1), Turkey (6), United Kingdom (17), and United States (131).

Cuba

Olga Sonia Leon Fernandez, *Havana*

Czech Republic

Vladimir Holan, *Prague*

Denmark

Klaus Gottlob Muller, *Copenhagen*

Finland

Andreas Scherer, *Kontiolahti*

France

Ignacio Anegon, *Nantes*
Guillaume Canaud, *Paris*
Felix Cantarovich, *Paris*
Roussel Jean Christian, *Nantes*
Bertrand Dupont, *Paris*
Loïc Fouillard, *Cergy-Pontoise*

Georgia

Archil Boris Chkhotua, *Tbilisi*

Germany

Elisenda Banon-Maneus, *Munchen*
Susanne Beckebaum, *Essen*
Andres Beiras-Fernandez, *Munich*
Rainer Birck, *Mannheim*
Hassan Dihazi, *Goettingen*
Christoph Eisenbach, *Heidelberg*
Frieder Keller, *Ulm*
Alfred Anton Konigsrainer, *Tuebingen*
Thomas Minor, *Bonn*
Peter Schemmer, *Heidelberg*
Meinolf Suttorp, *Dresden*
Rene H Tolba, *Aachen*
Wolfgang Wagner, *Aachen*
Min-Min Wang, *Berlin*

Greece

Costas Fourtounas, *Patras*
Evgenios Goussetis, *Athens*
Maria Koukoulaki, *Rion*
Sophia Lionaki, *Athens*
Anna Petropoulou, *Athens*
Alexandros Spyridonidis, *Patras*

Hungary

Andrea Ferencz, *Budapest*
Peter Hamar, *Budapest*

India

Sanjay Kumar Agarwal, *New Delhi*
Suraksha Agrawal, *Lucknow*
Biju George, *Tamilnadu*
Lalit Kumar, *New Delhi*
Pravin Mhatre, *Mumbai*
Geeta Ravindran, *Munbai*
Avnish Kumar Seth, *New Delhi*
Malancha Ta, *Bangalore*

Iran

Parisa Badiee, *Shiraz*
Seyed Mohsen Dehghani, *Shiraz*
Ahad Eshraghian, *Shiraz*
Ali Ghafari, *Urmia*
Mitra Mahdavi-Mazdeh, *Tehran*
Saeed Taheri, *Tehran*
Ramin Yaghobi, *Shiraz*

Israel

Esther Granot, *Jerusalem*
Assy Najib Nimer, *Safed*
Inna Sinuani, *Zerifin*
Shimon Slavin, *Tel Aviv*

Italy

Gian Luigi Adani, *Udine*
Pietro Andreone, *Bologna*
Umberto Baccarani, *Udine*
Bruno Bonetti, *Verona*
Alessandro Busca, *Turin*
Giovanni Camussi, *Turin*
Cristina Costa, *Turin*
Stefano Faenza, *Bologna*
Gian Marco Ghiggeri, *Genoa*
Grandaliano Giuseppe, *Foggia*
Andrea Giusti, *Genoa*
Paola Gremigni, *Bologna*
Walter Franco Grigioni, *Bologna*
Salvatore Gruttadauria, *Palermo*
Alessandro Isidori, *Pesaro*
Giampiero La Rocca, *Palermo*
Giovanni Landoni, *Milano*
Giovanni Li Volti, *Catania*
Renzo Mignani, *Rimini*
Luca Neri, *Milan*
Luciano Potena, *Bologna*
Matteo Ravaioli, *Bologna*
Giuseppe Remuzzi, *Bergamo*
Giulio Romano, *Udine*
Vito Ruggiero, *Rome*
Sergio Rutella, *Rome*
Fabrizio Sansone, *Turin*
Michele Santangelo, *Naples*
Antonino Sessa, *Naples*
Aurelio Sonzogni, *Bergamo*
Giovanni Stallone, *Foggia*
Lamponi Stefania, *Siena*
Giovanni Luigi Tripepi, *Reggio Calabria*
Cornelio Uderzo, *Milan*
Massimiliano Veroux, *Catania*

Japan

Yasuhiro Fujino, *Akashi*
Yoshitaka Isaka, *Osaka*
Junya Kanda, *Durham*
Hiroshi Kanno, *Yokohama*
Mureo Kasahara, *Tokyo*
Xiao-Kang Li, *Tokyo*
Shinichi Miyagawa, *Matsumoto*
Shugo Mizuno, *Tsu*
Takehiko Mori, *Tokyo*
Daisuke Morioka, *Yokohama*
Hirofumi Noguchi, *Okayama*
Masahiko Okamoto, *Kyoto*
Yasuhiko Sugawara, *Tokyo*
Shoichiro Sumi, *Kyoto*
Masahiko Taniguchi, *Asahikawa*
Shintaro Yamazaki, *Tokyo*
Kotaro Yoshimura, *Tokyo*
Katsutoshi Yoshizato, *Higashi-Hiroshima*
Kenji Yuzawa, *Ibaraki-ken*

Jordan

Mahmoud Mohammad Sarhan, *Amman*

Macedonia

Goce Spasovski, *Skopje*

Mexico

Rene Drucker-Colln, *Mexico City*
Gustavo Martinez-Mier, *Veracruz*

Morocco

Faissal Tarrass, *Larache*

Netherlands

Michiel GH Betjes, *Rotterdam*
Frank JMF Dor, *Rotteram*
Irma Joosten, *Nijmegen*
Bernard AJ Roelen, *Utrecht*
Luc JW van der Laan, *Rotterdam*

Nigeria

Anthony A Oyekunle, *Ile-Ife*

Norway

Lars Lysgaard Gullestad, *Oslo*

Pakistan

Tahir Sultan Shmasi, *Karachi*

Poland

Piotr Czubkowski, *Warsaw*
Andrzej Zbigniew Rydzewski, *Warszawa*

Qatar

Moutaz Farouk Derbala, *Doha*

Saudi Arabia

Ali Al-Ahmari, *Riyadh*
Imran Khalid, *Jeddah*
Mohamed Mabed, *Jeddah*
Mohamed M Sayed-Ahmed, *Riyadh*

Singapore

Seng Hock Quak, *Singapore*

South Korea

Curie Ahn, *Seoul*
Jong Wook Chang, *Seoul*
Baik Hwan Cho, *Jeonju City*
Hyeon Joo Jeong, *Seoul*
Koo-Jeong Kang, *Daegu*
Chang Nyung Kim, *Yongin*
Gaab Soo Kim, *Seoul*
Kyung Mo Kim, *Seoul*
Yon Su Kim, *Seoul*
Jong Wook Lee, *Seoul*
Sang-Oh Lee, *Seoul*
Kyo-Cheol Mun, *Daegu*
Eun-Jee Oh, *Seoul*
Kwon Moo Park, *Daegu*
Chul Woo Yang, *Seoul*
Kun-Ho Yoon, *Seoul*
Seungkwon You, *Seoul*

Spain

Manuel Molina Arias, *Madrid*
Ruben Ciria, *Cordoba*
Luis Fontana, *Granada*
Maria Marco, *Barcelona*
Alberto Ortiz, *Madrid*
Julio Pascual, *Barcelona*
Carmen Peralta, *Barcelona*
Jose Antonio Pons, *Murcia*
Jesus Vaquero, *Madrid*

Sweden

Tobias Larsson, *Stockholm*

Switzerland

Andrea De Gottardi, *Berne*
Michela G Tempia-Caliera Schappi, *Geneva*
Christian Toso, *Geneva*

Thailand

Suradej Hongeng, *Bangkok*
Kittisupamongkol Weekitt, *Bangkok*

Tunisia

Kais Harzallah, *Tunis*

Turkey

Elvan Caglar Citak, *Mersin*
Emir Baki Denkbas, *Ankara*
İhsan Ergün, *Ankara*
Murat Kilic, *Izmir*
Oner Ozdemir, *Istanbul*
Baris D Yildiz, *Ankara*

United Kingdom

Jacob Attah Akoh, *Plymouth*
Atul Bagul, *Leicester*
Ricky Harminder Bhogal, *Birmingham*
Richard John Borrows, *Birmingham*
Eric Chemla, *London*
Sarah Anne Hosgood, *Leicester*
Stefan Georg Hűbscher, *Birmingham*
Alireza Hamidian Jahromi, *London*
Alan Jardine, *Glasgow*
Sanjeev Kanoria, *London*
Michel Modo, *London*
Paolo Muiesan, *Birmingham*
GH Neild, *London*
Magdi Shehata, *Leicester*
Afshin Tavakoli, *Manchester*
Alexander Woywodt, *Preston*
Qihe Xu, *London*

United States

Arshak R Alexanian, *Milwaukee*
Sharif Ali, *Detroit*
Jaime Aranda-Michel, *Jacksonville*
Robert Aris, *Chapel Hill*
Reto M Baertschiger, *Indianapolis*
David A Baran, *Newark*
Gerald Brandacher, *Baltimore*
Joseph F Buell, *New Orleans*
Herman S Cheung, *Coral Gables*
Gaetano Ciancio, *Miami*
Diane M Cibrik, *Ann Arbor*
Luca Cicalese, *Galveston*
Ari Cohen, *New Orleans*
David KC Cooper, *Pittsburgh*
Darshana Manji Dadhania, *New York*
Graciela De Boccardo, *New York*
Cataldo Doria, *Philadelphia*
Amrita Dosanjh, *San Diego*
Stavros G Drakos, *Salt Lake*
Sukru Emre, *New Haven*
Sherif S Farag, *Indianapolis*
Roberto J Firpi, *Gainesville*
Robert A Fisher, *Richmond*
Amy L Friedman, *Syracuse*
Tibor Fulop, *Jackson*
G Ian Gallicano, *Washington*
Wenda Gao, *Boston*
Roberto Gedaly, *Lexington*
W Scott Goebel, *Indianapolis*
Rujun Gong, *Providence*
Chad R Gordon, *Boston*
Angelika C Gruessner, *Tucson*
Gregg Allen Hadley, *Columbus*
Jeffrey B Halldorson, *Seattle*
Mehdi Hamadani, *Morgantown*
Karen Hardinger, *Kansas*
Imed Helal, *Aurora*
Allan Duane Hess, *Baltimore*
Ibtesam A Hilmi, *Pittsburgh*
Andres Jaramillo, *Itasca*
Randeep Kashyap, *Rochester*
Tatsuo Kawai, *Boston*
Ajai Khanna, *San Diego*
Dean Y Kim, *Detroit*
Katsuhiro Kita, *Galveston*
David J Kramer, *Jacksonville*
Jerzy W Kupiec-Weglinski, Los Angeles
Paul Y Kwo, *Indianapolis*
Victor E Laubach, *Charlottesville*
Techung Lee, *Buffalo*
Josh Levitsky, *Chicago*
Xian C Li, *Boston*
Suthat Liangpunsakul, *Indianapolis*
Seah H Lim, *Amarillo*
Ching-Shwun Lin, *San Francisco*
Julie Lin, *Boston*
Delong Liu, *Westchester*
Andrew Leon Lobashevsky, *Indianapolis*
Paul Allen Lucas, *Valhalla*
Xunrong Luo, *Chicago*
Didier A Mandelbrot, *Boston*
Martin J Mangino, *Richmond*
Richard S Mangus, *Indianapolis*
Ignazio R Marino, *Philadelphia*
Paulo Ney Aguiar Martins, *Boston*
Andrew Scott Mathis, *Long Branch*
James Michael Millis, *Chicago*
Tamir Miloh, *Phoenix*
Ayse Leyla Mindikoglu, *Baltimore*
Amr El-Husseini Mohamed, *Lexington*
Sandeep Mukherjee, *Omaha*
Yoko Mullen, *Duarte*
Raghavan Murugan, *Pittsburgh*
Tibor Nadasdy, *Columbus*
Atsunori Nakao, *Pittsburgh*
Singh Neeraj, *Columbus*
Justin H Nguyen, *Jackosnville*
Volker Nickeleit, *Chapel Hill*
Christopher Niyibizi, *Hershey*
Macaulay Onuigbo, *Eau Claire*
Jorge Alberto Ortiz, *Philadelphia*
Antonello Pileggi, *Miami*
Raymond M Planinsic, *Pittsburgh*
Qi Qian, *Rochester*
Rajalingam Raja, *Los Angeles*
Michael A Ramsay, *Dallas*
Raymund Rabe Razonable, *Rochester*
Mohammed S Razzaque, *Boston*
Pavan Reddy, *Ann Arbor*
Camillo Ricordi, *Miami*
Horacio L Rilo, *Tucson*
David Alan Rizzieri, *Durham*
Simon C Robson, *Boston*
Alvaro Rojas-Pena, *Ann Arbor*
Kenneth Rolston, *Houston*

World Journal of Transplantation

Contents

Quarterly Volume 3 Number 4 December 24, 2013

AIM AND SCOPE

World Journal of Transplantation (*World J Transplant*, *WJT*, online ISSN 2220-3230, DOI: 10.5500) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJT covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, and xenotransplantation. The current columns of *WJT* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

AIM AND SCOPE

World Journal of Transplantation is now indexed in PubMed Central, PubMed, Digital Object Identifier.

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Quarterly

EDITOR-IN-CHIEF
Maurizio Salvadori, MD, Professor, Renal Unit, Careggi University Hospital, Viale Pieraccini 18, Florence 50139, Italy

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director

World Journal of Transplantation
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road, Wanchai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
December 24, 2013

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2220-3230/g_info_20100722180909.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.48

World J Transplant 2013 December 24; 3(4): 48-53
ISSN 2220-3230 (online)

EDITORIAL

Current status of clinical islet transplantation

Andrew R Pepper, Boris Gala-Lopez, Oliver Ziff, AM James Shapiro

Andrew R Pepper, Boris Gala-Lopez, AM James Shapiro, Department of Clinical Islet Transplant Program, University of Alberta, Edmonton T6G 2C8, Alberta, Canada
Oliver Ziff, AM James Shapiro, Department of Surgery, University of Alberta, Edmonton T6G 2B7, Alberta, Canada
Author contributions: Pepper AR and Gala-Lopez B contributed equally to this work designing the research and writing the paper; Ziff O performed bibliographic revision and designed figures; Shapiro AMJ designed the research and revised the final manuscript as senior author.
Correspondence to: AM James Shapiro, MD, PhD, Professor, Director of Clinical Islet and Living Donor Liver Transplant Programs, Department of Clinical Islet Transplant Program, University of Alberta, 2000 College Plaza, 8215-112th St, Edmonton T6G 2C8, Alberta, Canada. amjs@islet.ca
Telephone: +1-780-4077330 Fax: +1-780-4078259
Received: July 26, 2013 Revised: August 16, 2013
Accepted: August 28, 2013
Published online: December 24, 2013

Abstract

Islet transplantation (IT) is today a well-established treatment modality for selected patients with type 1 diabetes mellitus (T1DM). After the success of the University of Alberta group with a modified approach to the immune protection of islets, the international experience grew along with the numbers of transplants in highly specialized centers. Yet, long-term analysis of those initial results from the Edmonton group indicated that insulin-independence was not durable and most patients return to modest amounts of insulin around the fifth year, without recurrent hypoglycemia events. Many phenomena have been identified as limiting factor for the islet engraftment and survival, and today all efforts are aimed to improve the quality of islets and their engrafting process, as well as more optimized immunosuppression to facilitate tolerance and ultimately, better long term survival. This brief overview presents recent progress in IT. A concise historical perspective is provided, along with the latest efforts to improve islet engraftment, immune protection and ultimately, prolonged graft survival. It is apparent that as the community continues to work together further optimizing IT, it is hopeful a cure for T1DM will soon be achievable.

Key words: Islet transplantation; Type 1 diabetes; Immunosuppression

Core tip: Since the initial inception of the "Edmonton protocol", phenomenal progress has transpired in the last decade. These milestones were namely due to the implementation of numerous pre-clinical and clinical investigations, testing innovative agents allowing potent immunotolerance with minimal complications as well as alternative transplant sites to overcome limitations inherent to the current intraportal access. As a result nearly 80% of full or partial graft function, out of more than 300 transplants performed to date. As the field of continues to work and progress together, it is foreseeable that a cure for type 1 diabetes mellitus is obtainable in the near future.

Pepper AR, Gala-Lopez B, Ziff O, Shapiro AMJ. Current status of clinical islet transplantation. *World J Transplant* 2013; 3(4): 48-53 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/48.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.48

INTRODUCTION

Islet transplantation (IT) is today an accepted modality to treat selected diabetic patients with frequent hypoglycemics and severe glycemic lability[1,2]. The "Edmonton Protocol" became a milestone by reporting sustained C-peptide production and high rates of insulin-independence after transplant in type 1 diabetes mellitus (T1DM)[3]. This reality became possible with the use of newer, more potent immunosuppressant (IS) agents, the avoidance of corticosteroids, and high-quality islet preparations, al-

though typically two islet infusions were required to attain insulin independence.

Long-term analysis of these initial results from the Edmonton group indicated that insulin-independence was not durable and most patients return to moderate amounts of insulin approximately 5 years post-infusion, in the absences of recurrent hypoglycemia events[4,5].

Causes for this chronic graft function remain unclear, but are likely associated with immune rejection, recurrence of autoimmunity or chronic exposure to diabetogenic IS agents[5,6].

This brief overview presents recent progress in IT. A succinct historical viewpoint is provided, along with the resent efforts to improve islet engraftment, immune protection and ultimately, prolonged graft survival.

HISTORICAL PERSPECTIVE

The history of IT is filled with numerous sacrifices and hardly fought successes. Early rudimentary experiments in the 19th Century lead to the concept of isolation and purification[5]. In 1966, the University of Minnesota group performed the first clinical attempt to cure T1DM by whole pancreas transplant[7,8]. It allowed technical improvements, but more importantly, refinements in IS while introducing cyclosporine continued with the use of multiple and more potent drug schemes.

Clinical investigators at Washington University demonstrated the possibility of reversing diabetes with temporary insulin independence after transplantation of human islets. It was a transient success because IS was still insufficient[9]. A year later, the first successful series of human islet allografts was reported by the University of Pittsburgh, achieving prolonged insulin-independence with a more optimized IS based on the recently introduced agent FK-506 and no steroids[9].

Another important milestone was the report from the University of Alberta group showing successful long-term results on selected patients, with the use of a novel IS scheme. Grafts were non-human leukocyte antigen (HLA) matched, patients were not sensitized (negative panel reactive antibody pre-transplant), islets were ABO compatible, and sequential transplants were used to deliver an adequate islet infusion mass by a percutaneous portal venous access route. Immunosuppression was tailored to avoid steroids and minimize calcineurin inhibitors to prevent diabetogenicity, with the combination of sirolimus, low-dose tacrolimus (TAC), and the daclizumab induction[10].

New programs proliferated worldwide based on the lessons learned from the "Edmonton Protocol" and the number of transplant significantly increased over the coming years. However, insulin independence was not durable long-term and most patient returned to modest amounts of insulin without risk of recurrent hypoglycemia by the third to fifth year. Additionally, approximately 25% required additional late islet infusions during the second or third year post-transplant[2].

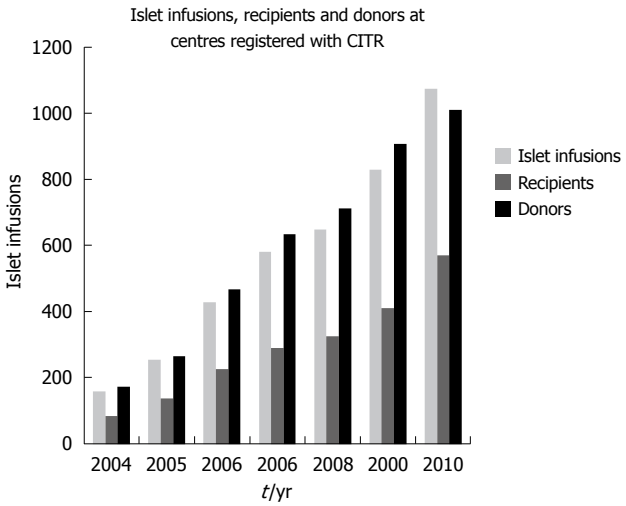

Figure 1 Total number of recent islet allograft infusion per recipient per donor in CITR-participating centers (2004-2010). Data adapted from 2010 CITR Seventh Annual Report.

New efforts are now aimed to improve the quality of islets, enhanced their engraftment conditions and prolonged their function. Moreover, new transplant sites are also consider overcoming the limitations of the traditional intraportal site and providing a suitable framework for future strategies, such as the use of insulin-producing stem cells as surrogate for the precious and increasingly scarce human islets.

EDMONTON'S CURRENT RESULTS

Despite significant improvements in the care of T1DM patients, a subgroup remains in significant disadvantage due to refractory hypoglycemia. The option of IT offers the possibility of improved glycemic control[2]. The recent years have witnessed substantial progress in the number and results of IT (Figure 1).

Before the year 2000, few centers performing IT achieved high rates of sustainable insulin independence after this procedure[2]. In 2000, Shapiro *et al*[3] reported their initial findings in seven consecutive subjects treated with glucocorticoid-free immunosuppressive therapy combined with infusion of an adequate mass of freshly prepared, bringing a new perspective on the immunoprotection provided for these patients[3]. The success achieved with this new scheme prompted interest and enthusiasm among various programs and launched a major international trial with key results for our current concepts on immunosuppression.

Today, the Clinical Islet Transplant Program at the University of Alberta remains as one of the most important and active transplant center in the world after becoming a beacon with one of the most integral and successful approaches to IT with sustained and reproducible long-term results. The task remains improving the viability of islet preparations and also in determining the most optimal IS agents to improve the initial results published

Table 1 Summary of current open clinical trials with interventions in islet transplantation (Adapted from Clinical Trials.gov)

Trial ID	Description	Institution
NCT01653899	Caspase Inhibition in Islet Transplantation	University of Alberta
NCT00468117	Efficacy of Islet After Kidney Transplantation	National Institute of Allergy and Infectious Diseases
NCT01705899	Islet Allotransplantation in Type 1 Diabetes	Ohio State University
NCT01652911	A Phase Ⅰ / Ⅱ Study of the Safety and Efficacy of Sernova's Cell Pouch™ for Therapeutic Islet Transplantation	University of Alberta
NCT00784966	Islet After Kidney Transplant for Type 1 Diabetes	Virginia Commonwealth University
NCT00790257	Safety and Efficacy Study of Encapsulated Human Islets Allotransplantation to Treat Type 1 Diabetes	Cliniques universitaires Saint-Luc-Université Catholique de Louvain
NCT00853944	Effect of Sitagliptin on Graft Function Following Islet Transplantation	University of British Columbia
NCT00249652	Transplant and Addiction Project 1	National Institute on Drug Abuse
NCT00530686	Pancreatic Islet Cell Transplantation - A Novel Approach to Improve Islet Quality and Engraftment	Baylor Research Institute
NCT01123187	Islet Cell Transplantation in Patients With Type 1 Diabetes With Previous Kidney Transplantation	University Hospital, Lille
NCT01817959	Study to Assess Efficacy and Safety of Reparixin in Pancreatic Islet Transplantation	Dompé s.p.a.
NCT00679042	Islet Transplantation in Type 1 Diabetic Patients Using the University of Illinois at Chicago Protocol	University of Illinois
NCT00453817	Islet of Langerhans Graft Monitoring by Magnetic Resonance Imaging	University Hospital, Geneva
NCT00853424	A Comparison of Islet Cell Transplantation With Medical Therapy for the Treatment of Diabetic Eye Disease	University of British Columbia
NCT00789308	Safety and Effectiveness of Low Molecular Weight Sulfated Dextran in Islet Transplantation	National Institute of Allergy and Infectious Diseases
NCT01148680	Trial Comparing Metabolic Efficiency of Islet Graft to Intensive Insulin Therapy for Type 1 Diabetes's Treatment	University Hospital, Grenoble
NCT01241864	Islet Transplantation in Type 1 Diabetic Kidney Allograft	University of Chicago
NCT01722682	Bone Marrow vs Liver as Site for Islet Transplantation	Ospedale San Raffaele
NCT01630850	Islet Transplantation in Patients With\Brittle\"Type 1 Diabetes"	University of Chicago
NCT01186562	Sitagliptin Therapy to Improve Outcomes After Islet Autotransplant	University of Minnesota
NCT01285934	A Trial of High Dose Immunosuppression and Autologous Hematopoietic Stem Cell Support Versus Intensive Insulin Therapy in Adults With Early Onset T1DM	University of Sao Paulo General Hospital
NCT00646724	Cotransplantation of Islet and Mesenchymal Stem Cell in Type 1 Diabetic Patients	Fuzhou General Hospital
NCT01379729	Bet Cell Therapy in Diabetes Type 1	AZ-VUB
NCT01341899	Efficacy and Safety Study of Autologous Hematopoietic Stem Cell Transplantation to Treat New Onset Type 1 Diabetes	The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
NCT01736228	Open-label Investigation of the Safety and Efficacy of DIABECELL in Patients With T1DM	Living Cell Technologies
NCT01346098	Islet Autotransplantation in Patients at Very High-risk Pancreatic Anastomosis	Ospedale San Raffaele
NCT00989547	Cord Blood Infusion for T1DM	Technische Universität München
NCT00807651	Autologous Hematopoietic Stem Cell Transplantation for Early Onset Type 1 Diabetes	Shanghai Jiao Tong University School of Medicine
NCT01042301	Profiling of Original Cellular and Humoral Biomarkers of Type 1 Diabetes	Nantes University Hospital
NCT01350219	Stem Cell Educator Therapy in Type 1 Diabetes	Tianhe Stem Cell Biotechnologies Inc.
NCT00014911	Immunosuppressive Medications for Participants in ITN005CT	National Institute of Allergy and Infectious Diseases

with the "Edmonton Protocol", but also to achieve single donor insulin-independence, safety and tolerability.

A recent study published a cross-sectional analysis of the current Edmonton results. It showed 79% of full or partial graft function out of more than 300 transplants performed. The median duration of insulin independence was 34.6 and 11.0 mo for subjects with full or partial graft function, whereas the duration of C-peptide was 53.3 and 70.4 mo for those same patients[11].

Phenomenal progress has occurred in the last years due to the implementation of numerous findings from preclinical and clinical investigations testing different agents to allow better immunotolerance with lesser complications, novel devices to provide islets with a safer environment, as well as new transplant sites to overcome limitations inherent to the current intraportal access (Table 1).

MAIN CHALLENGES TO IT

It is apparent that in light of the therapeutic advantages of β-cell replacement through IT, numerous contributing factors hinder islet graft survival and function. These obstacles must be overcome in order for this therapy to become the ubiquitous alternative to pancreas transplantation and exogenous insulin administration. Despite intrahepatic islet infusion being the route of choice for over three decades[12,13] in both experimental and clinical settings, several complications with this approach exist which may account for islet graft attrition[14-18]. The liver indeed has an advantage of a multiple vascular supply, however, its parenchymal oxygen tension, is well below that of the pancreas and is not conducive to islet survival[19,20]. Furthermore, the infusion of islets into the liver

is associated with inherit procedural risks including but not limited to catheter-induced hemorrhage and thrombosis[21]. Disadvantages of this route of islet administration also include limited ability to image islet grafts post-transplant, incapacity to retrieve the graft if required, and restricted quantity of β-cell mass that can recipient can receive due to portal pressure elevation[14-18,21,22]. The innate immune system further contributes to a reduction in β cells mass acutely post-infusion into the patient's portal circulation. It is estimated that greater than 50% of the transplanted islets are lost within hours post infusion which is thought in part to be due to the immediate blood mediated inflammatory reaction and complement coagulation cascade, as evidenced by acute C-peptide release, and from quantitative positron emission tomography scan imagery[14-16,23-27]. These factors in conjunction with the diabetogenic action of the immunosuppressive drugs [*i.e.*, calcineurin inhibits, sirolimus, mycophenolate mofetil (MMF)][28], suboptimal islet revascularization[29,30], both the adaptive and innate immune responses, potential HLA-antigen[31-34] sensitization and lack of an effective means of determining islet potency prior to transplant, together contribute to an inept utilization of the small number of available cadaveric donor pancreata[16,17]. The difficulties and limitations associated with hepatic portal vein infusion have stimulated robust efforts into investigation strategies to improve islet engraftment, such as refined IS protocols, surrogate sources of β-cells (*i.e.*, stem cells or porcine islets) and alternative transplantation sites, in effort to increase the potential for long-term islet graft survival and function[16,18].

IMPROVING ISLET GRAFT FUNCTION

The early results from IT should be taken into context and compared to alternative treatments modalities. In contrast to pancreas transplantation, IT is still in its infancy. Roughly 750 type 1 diabetic patients have received an IT among the some 30 active international islet centers over the past decade. In comparison, approximately 30000 pancreas transplants have been conducted over the past three decades[35-38]. Despite the relatively low number of islet recipients, encouraging results with IT have recently been reported, such as the greater than 50% insulin-independent rates from solitary pancreas transplantation 5 years post-transplant has now been matched by IT in at least four independent centers, namely Edmonton, Minneapolis, Genva and Lille. It is evident that recent significant advances in islet preparation and immunosuppressive therapy have improved the efficacy and safety of IT to the point that it now challenges whole organ pancreas transplantation.

Due to the multiple pathways known to be involved in β-cell attrition, including the autoreactive and alloreactive immune response, as well as the alloresponse it can be argued that a monotherapy IS approach is improbable to further enhance IT outcomes. Indeed, strategies towards single-donor IT has begun by implementing multiple pathways blockades to IS cocktails, which face the challenges of promoting islet graft survival. Combining anti-inflammatory biologics to maintenance IS have led to improved single-donor success rates at the University of Minnesota[39,40]. The success rate of islet donor islet recipients has dramatically increased from 10%-40% when peritransplant insulin and heparin intervention has been employed[27]. Tumor necrosis factor-α blockage by etanercept has improved single-donor islet transplant outcomes as well[27,40-44]. In preclinical settings specific anti-inflammatory agents such as the interleukin-1 receptor antagonist anakinra and etanercept significantly increased marginal mass islet engraftment[41-45]. Furthermore, anti-apoptosis and growth stimulation [*i.e.*, glucagon-like peptide 1 (GLP-1)] have further demonstrated advantageous results in both preclinical and clinical studies, for instance the short acting GLP-1 analogue exenatide demonstrated an increased single-donor islet engraftment success rate[46-48]. Clonal depletion of alloreactive T cells appears promote a hyporesponsive environment and peripheral mechanisms of anergy, thus driving the shift towards tolerance[49,50]. The use of T-cell depletion induction methods such as alemtuzumab in conjunction with TAC/MMF have resulted in substantial improvements in long-term insulin-independence (> 5 years)[51,52]. In addition, a current example of the extraordinary progress that has been made when combine IS strategies are implemented, is the remarkable success that has been achieved when co-stimulation blockage using belatacept (inhibiting CD80-CD86 interactions) in conjunction with T-cell depletion induction and in the absence of calcineurin inhibitors led to insulin independence with islets from a single donor and prolonged allograft survival[6,53]. It is clear current immunosuppressive therapies have become well tolerated and safer for the recipient by minimizing the adverse side effects while improving islet engraftment.

CONCLUSION

Since the first pioneering experimental and clinical studies, substantial improvements have been made in IT, leading to the development of the "Edmonton Protocol". Over the past decade, since this protocols inception, continued progress in the field has resulted in markedly higher rates of single-islet recipient success rates as well as sustained insulin-independences (> 5 years). Not to be forgotten are the benefits for microvascular complications (*i.e.*, reduced retinopathy) and the amelioration of hypoglycemic unaware events following IT, in most cases irrespective of glycemic control. Despite the favourable long-term safety profile associated with IT, many unanswered questions still exist; namely, the causality of islet graft function and attrition. For instance reduction in HbA1C and hypoglycaemia normally attributed to graft function may in part be a reflection of close glycemic monitoring. Equally, graft dysfunction and poor glycemic control post-transplant may be attributed to poor adher-

ence and psychosocial influences among others, rather than exclusively caused by islet graft loss[54]. Some of these answers may very well indeed be answered through randomized clinical trials. By no means should IT be perceived as a cure for all type 1 diabetics, however for a subset of individuals with severe glycemic lability, IT has been demonstrated to be an excellent therapeutic strategy to achieve glycemic control and abrogation of hypoglycaemia. As the field of continues to work and progress together, in effort to refine and optimized IT, it is foreseeable that a cure for T1DM is obtainable in the not so distant future.

REFERENCES

1 **Ryan EA**, Bigam D, Shapiro AM. Current indications for pancreas or islet transplant. *Diabetes Obes Metab* 2006; **8**: 1-7 [PMID: 16367876 DOI: 10.1111/j.1463-1326.2004.00460.x]

2 **Shapiro AM**, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006; **355**: 1318-1330 [PMID: 17005949 DOI: 10.1056/NEJMoa061267]

3 **Shapiro AM**, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230-238 [PMID: 10911004 DOI: 10.1056/NEJM200007273430401]

4 **Jahansouz C**, Jahansouz C, Kumer SC, Brayman KL. Evolution of β-cell replacement therapy in diabetes mellitus: Islet cell transplantation. *J Transplant* 2011; **2011**: 247959 [PMID: 22013505 DOI: 10.1155/2011/247959]

5 **Shapiro J**, Shaw J. A historical perspective on experimental and clinical islet transplantation. In: Shapiro AMJ, Shaw JAM, editors. Islet transplantation and beta cell replacement therapy. New York: Informa Healthcare, 2007: 1-28

6 **Gala-Lopez B**, Pepper AR, Shapiro AM. Biologic agents in islet transplantation. *Curr Diab Rep* 2013; **13**: 713-722 [PMID: 23918618 DOI: 10.1007/s11892-013-0414-8]

7 **Squifflet JP**, Gruessner RW, Sutherland DE. The history of pancreas transplantation: past, present and future. *Acta Chir Belg* 2008; **108**: 367-378 [PMID: 18710120]

8 **Han DJ**, Sutherland DE. Pancreas transplantation. *Gut Liver* 2010; **4**: 450-465 [PMID: 21253293 DOI: 10.5009/gnl.2010.4.4.450]

9 **Ricordi C**, Strom TB. Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol* 2004; **4**: 259-268 [PMID: 15057784 DOI: 10.1038/nri1332]

10 **Ryan EA**, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; **54**: 2060-2069 [PMID: 15983207]

11 **Senior PA**, Kin T, Shapiro J, Koh A. Islet transplantation at the University of Alberta: Status update and review of progress over the last decade. *Can J Diabetes* 2012; **36**: 32-37

12 **Scharp DW**, Murphy JJ, Newton WT, Ballinger WF, Lacy PE. Transplantation of islets of Langerhans in diabetic rhesus monkeys. *Surgery* 1975; **77**: 100-105 [PMID: 122797]

13 **Kemp CB**, Scharp DW, Knight MJ, Ballinger WF, Lacy PE. Importance of implantation site of pancreatic islet isografts in treatment of experimental diabetes. *Surg Forum* 1973; **24**: 297-299 [PMID: 4282521]

14 **Korsgren O**, Nilsson B. Improving islet transplantation: a road map for a widespread application for the cure of persons with type I diabetes. *Curr Opin Organ Transplant* 2009; **14**: 683-687 [PMID: 19779341 DOI: 10.1097/MOT.0b013e328332c44c]

15 **Korsgren O**, Lundgren T, Felldin M, Foss A, Isaksson B, Permert J, Persson NH, Rafael E, Rydén M, Salmela K, Tibell A, Tufveson G, Nilsson B. Optimising islet engraftment is critical for successful clinical islet transplantation. *Diabetologia* 2008; **51**: 227-232 [PMID: 18040664 DOI: 10.1007/s00125-007-0868-9]

16 **Harlan DM**, Kenyon NS, Korsgren O, Roep BO. Current advances and travails in islet transplantation. *Diabetes* 2009; **58**: 2175-2184 [PMID: 19794074 DOI: 10.2337/db09-0476]

17 **Rother KI**, Harlan DM. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. *J Clin Invest* 2004; **114**: 877-883 [PMID: 15467822 DOI: 10.1172/JCI23235]

18 **Merani S**, Toso C, Emamaullee J, Shapiro AM. Optimal implantation site for pancreatic islet transplantation. *Br J Surg* 2008; **95**: 1449-1461 [PMID: 18991254 DOI: 10.1002/bjs.6391]

19 **Carlsson PO**, Palm F, Andersson A, Liss P. Chronically decreased oxygen tension in rat pancreatic islets transplanted under the kidney capsule. *Transplantation* 2000; **69**: 761-766 [PMID: 10755523]

20 **Carlsson PO**, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 2001; **50**: 489-495 [PMID: 11246867]

21 **Kawahara T**, Kin T, Kashkoush S, Gala-Lopez B, Bigam DL, Kneteman NM, Koh A, Senior PA, Shapiro AM. Portal vein thrombosis is a potentially preventable complication in clinical islet transplantation. *Am J Transplant* 2011; **11**: 2700-2707 [PMID: 21883914 DOI: 10.1111/j.1600-6143.2011.03717.x]

22 **Kawahara T**, Kin T, Shapiro AM. A comparison of islet autotransplantation with allotransplantation and factors elevating acute portal pressure in clinical islet transplantation. *J Hepatobiliary Pancreat Sci* 2012; **19**: 281-288 [PMID: 21879320 DOI: 10.1007/s00534-011-0441-2]

23 **Bennet W**, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, Bretzel RG, Elgue G, Larsson R, Nilsson B, Korsgren O. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes* 1999; **48**: 1907-1914 [PMID: 10512353]

24 **Bennet W**, Groth CG, Larsson R, Nilsson B, Korsgren O. Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. *Ups J Med Sci* 2000; **105**: 125-133 [PMID: 11095109]

25 **Eriksson O**, Eich T, Sundin A, Tibell A, Tufveson G, Andersson H, Felldin M, Foss A, Kyllönen L, Langstrom B, Nilsson B, Korsgren O, Lundgren T. Positron emission tomography in clinical islet transplantation. *Am J Transplant* 2009; **9**: 2816-2824 [PMID: 19845588 DOI: 10.1111/j.1600-6143.2009.02844.x]

26 **Eich T**, Eriksson O, Lundgren T. Visualization of early engraftment in clinical islet transplantation by positron-emission tomography. *N Engl J Med* 2007; **356**: 2754-2755 [PMID: 17596618 DOI: 10.1056/NEJMc070201]

27 **Koh A**, Senior P, Salam A, Kin T, Imes S, Dinyari P, Malcolm A, Toso C, Nilsson B, Korsgren O, Shapiro AM. Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. *Transplantation* 2010; **89**: 465-471 [PMID: 20177350 DOI: 10.1097/TP.0b013e3181c478fd]

28 **Johnson JD**, Ao Z, Ao P, Li H, Dai LJ, He Z, Tee M, Potter KJ, Klimek AM, Meloche RM, Thompson DM, Verchere CB, Warnock GL. Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. *Cell Transplant* 2009; **18**: 833-845 [PMID: 19500470 DOI: 10.3727/096368909X471198]

29 **Brissova M**, Fowler M, Wiebe P, Shostak A, Shiota M, Radhika A, Lin PC, Gannon M, Powers AC. Intraislet endothelial

cells contribute to revascularization of transplanted pancreatic islets. *Diabetes* 2004; **53**: 1318-1325 [PMID: 15111502]
30 **Brissova M**, Powers AC. Revascularization of transplanted islets: can it be improved? *Diabetes* 2008; **57**: 2269-2271 [PMID: 18753672 DOI: 10.2337/db08-0814]
31 **Campbell PM**, Senior PA, Salam A, Labranche K, Bigam DL, Kneteman NM, Imes S, Halpin A, Ryan EA, Shapiro AM. High risk of sensitization after failed islet transplantation. *Am J Transplant* 2007; **7**: 2311-2317 [PMID: 17845564 DOI: 10.1111/j.1600-6143.2007.01923.x]
32 **Campbell PM**, Salam A, Ryan EA, Senior P, Paty BW, Bigam D, McCready T, Halpin A, Imes S, Al Saif F, Lakey JR, Shapiro AM. Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. *Am J Transplant* 2007; **7**: 1242-1248 [PMID: 17456201 DOI: 10.1111/j.1600-6143.2007.01777.x]
33 **Naziruddin B**, Wease S, Stablein D, Barton FB, Berney T, Rickels MR, Alejandro R. HLA class I sensitization in islet transplant recipients: report from the Collaborative Islet Transplant Registry. *Cell Transplant* 2012; **21**: 901-908 [PMID: 22080832 DOI: 10.3727/096368911X612468]
34 **Jackson AM**, Connolly JE, Matsumoto S, Noguchi H, Onaca N, Levy MF, Naziruddin B. Evidence for Induced Expression of HLA Class II on Human Islets: Possible Mechanism for HLA Sensitization in Transplant Recipients. *Transplantation* 2009; **87**: 500-506 [PMID: 19307785 DOI: 10.1097/TP.0b013e318195fc33]
35 **Shapiro AM**. State of the art of clinical islet transplantation and novel protocols of immunosuppression. *Curr Diab Rep* 2011; **11**: 345-354 [PMID: 21830042 DOI: 10.1007/s11892-011-0217-8]
36 **Kandaswamy R**, Sutherland DE. Pancreas versus islet transplantation in diabetes mellitus: How to allocate deceased donor pancreata? *Transplant Proc* 2006; **38**: 365-367 [PMID: 16549122 DOI: 10.1016/j.transproceed.2006.01.005]
37 **Sutherland DE**, Gruessner RW, Dunn DL, Matas AJ, Humar A, Kandaswamy R, Mauer SM, Kennedy WR, Goetz FC, Robertson RP, Gruessner AC, Najarian JS. Lessons learned from more than 1,000 pancreas transplants at a single institution. *Ann Surg* 2001; **233**: 463-501 [PMID: 11303130]
38 **Sutherland DE**, Gruessner RW, Gruessner AC. Pancreas transplantation for treatment of diabetes mellitus. *World J Surg* 2001; **25**: 487-496 [PMID: 11344403 DOI: 10.1007/s002680020342]
39 **Hering BJ**. Repurification: rescue rather than routine remedy. *Am J Transplant* 2005; **5**: 1-2 [PMID: 15636604 DOI: 10.1111/j.1600-6143.2004.00760.x]
40 **Hering BJ**, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DE. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 2005; **293**: 830-835 [PMID: 15713772 DOI: 10.1001/jama.293.7.830]
41 **Matsumoto S**, Takita M, Chaussabel D, Noguchi H, Shimoda M, Sugimoto K, Itoh T, Chujo D, SoRelle J, Onaca N, Naziruddin B, Levy MF. Improving efficacy of clinical islet transplantation with iodixanol-based islet purification, thymoglobulin induction, and blockage of IL-1β and TNF-α. *Cell Transplant* 2011; **20**: 1641-1647 [PMID: 21396171 DOI: 10.3727/096368910X564058]
42 **Shapiro AM**, Ricordi C. Unraveling the secrets of single donor success in islet transplantation. *Am J Transplant* 2004; **4**: 295-298 [PMID: 14961980]
43 **Xenos ES**, Farney AC, Widmer MB, Casanova D, Stevens RB, Blazar BR, Sutherland DE, Gores PF. Effect of tumor necrosis factor alpha and of the soluble tumor necrosis factor receptor on insulin secretion of isolated islets of Langerhans. *Transplant Proc* 1992; **24**: 2863-2864 [PMID: 1334599]
44 **Rewers M**, Gottlieb P. Immunotherapy for the prevention and treatment of type 1 diabetes: human trials and a look into the future. *Diabetes Care* 2009; **32**: 1769-1782 [PMID: 19794002 DOI: 10.2337/dc09-0374]
45 **McCall M**, Pawlick R, Kin T, Shapiro AM. Anakinra potentiates the protective effects of etanercept in transplantation of marginal mass human islets in immunodeficient mice. *Am J Transplant* 2012; **12**: 322-329 [PMID: 22053751 DOI: 10.1111/j.1600-6143.2011.03796.x]
46 **Cechin SR**, Pérez-Álvarez I, Fenjves E, Molano RD, Pileggi A, Berggren PO, Ricordi C, Pastori RL. Anti-inflammatory properties of exenatide in human pancreatic islets. *Cell Transplant* 2012; **21**: 633-648 [PMID: 21669040 DOI: 10.3727/096368911X576027]
47 **Faradji RN**, Froud T, Messinger S, Monroy K, Pileggi A, Mineo D, Tharavanij T, Mendez AJ, Ricordi C, Alejandro R. Long-term metabolic and hormonal effects of exenatide on islet transplant recipients with allograft dysfunction. *Cell Transplant* 2009; **18**: 1247-1259 [PMID: 20003758 DOI: 10.3727/096368909X474456]
48 **Ghofaili KA**, Fung M, Ao Z, Meloche M, Shapiro RJ, Warnock GL, Elahi D, Meneilly GS, Thompson DM. Effect of exenatide on beta cell function after islet transplantation in type 1 diabetes. *Transplantation* 2007; **83**: 24-28 [PMID: 17220786 DOI: 10.1097/01.tp.0000251379.46596.2d]
49 **Bhatt S**, Fung JJ, Lu L, Qian S. Tolerance-inducing strategies in islet transplantation. *Int J Endocrinol* 2012; **2012**: 396524 [PMID: 22675353 DOI: 10.1155/2012/396524]
50 **Sutherland DE**, Radosevich DM, Bellin MD, Hering BJ, Beilman GJ, Dunn TB, Chinnakotla S, Vickers SM, Bland B, Balamurugan AN, Freeman ML, Pruett TL. Total pancreatectomy and islet autotransplantation for chronic pancreatitis. *J Am Coll Surg* 2012; **214**: 409-424; discussion 424-426 [PMID: 22397977 DOI: 10.1016/j.jamcollsurg.2011.12.040]
51 **Shapiro AM**. Strategies toward single-donor islets of Langerhans transplantation. *Curr Opin Organ Transplant* 2011; **16**: 627-631 [PMID: 22068022 DOI: 10.1097/MOT.0b013e32834cfb84]
52 **Froud T**, Baidal DA, Faradji R, Cure P, Mineo D, Selvaggi G, Kenyon NS, Ricordi C, Alejandro R. Islet transplantation with alemtuzumab induction and calcineurin-free maintenance immunosuppression results in improved short- and long-term outcomes. *Transplantation* 2008; **86**: 1695-1701 [PMID: 19104407 DOI: 10.1097/TP.0b013e31819025e5]
53 **Posselt AM**, Szot GL, Frassetto LA, Masharani U, Tavakol M, Amin R, McElroy J, Ramos MD, Kerlan RK, Fong L, Vincenti F, Bluestone JA, Stock PG. Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade. *Transplantation* 2010; **90**: 1595-1601 [PMID: 20978464 DOI: 10.1097/TP.0b013e3181fe1377]
54 **Barton FB**, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, Oberholzer J, Odorico JS, Garfinkel MR, Levy M, Pattou F, Berney T, Secchi A, Messinger S, Senior PA, Maffi P, Posselt A, Stock PG, Kaufman DB, Luo X, Kandeel F, Cagliero E, Turgeon NA, Witkowski P, Naji A, O'Connell PJ, Greenbaum C, Kudva YC, Brayman KL, Aull MJ, Larsen C, Kay TW, Fernandez LA, Vantyghem MC, Bellin M, Shapiro AM. Improvement in outcomes of clinical islet transplantation: 1999-2010. *Diabetes Care* 2012; **35**: 1436-1445 [PMID: 22723582 DOI: 10.2337/dc12-0063]

P- Reviewers: Chaib E, Fulop T **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Zheng XM

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.54

World J Transplant 2013 December 24; 3(4): 54-61
ISSN 2220-3230 (online)

EDITORIAL

Common and small molecules as the ultimate regulatory and effector mediators of antigen-specific transplantation reactions

Vladimir Holan, Magdalena Krulova

Vladimir Holan, Magdalena Krulova, Department of Transplantation Immunology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, 142 20 Prague 4, Czech Republic
Vladimir Holan, Magdalena Krulova, Faculty of Science, Charles University, 128 44 Prague 2, Czech Republic
Author contributions: All authors contributed equally to this work.
Supported by The Grants P304/11/0653 and P301/11/1568 from the Grant Agency of the Czech Republic; the Grant NT/14102 from the Grant Agency of the Ministry of Health of the Czech Republic; and the projects MSM0021620858 and SVV 265211 from the Ministry of Education of the Czech Republic
Correspondence to: Vladimir Holan, PhD, DSc, Professor of Immunology, Department of Transplantation Immunology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic. holan@biomed.cas.cz
Telephone: +420-241-063226 Fax: +420-224-432699
Received: March 15, 2013 Revised: June 12, 2013
Accepted: August 8, 2013
Published online: December 24, 2013

Abstract

In spite of intensive research, the molecular basis of allograft and xenograft rejection still remains not fully understood. The acute rejection of an allograft is associated with the intragraft Th1 cytokine response, while tolerance of an allograft or xenograft rejection is accompanied by a higher production of the Th2 cytokines interleukin (IL)-4 and IL-10. Nevertheless, these cytokines are not the final regulatory and effector molecules mediating transplantation reactions. Data indicate that the functioning of common molecules with enzymatic activities, such are inducible nitric oxide synthase (iNOS), arginase, heme oxygenase-1 (HO-1) or indoleamine-2,3-dioxygenase (IDO), the bioavailability of their substrates (L-arginine, tryptophan, heme) and the cytotoxic and regulatory actions of their small gaseous products (NO, CO) can be the ultimate mechanisms responsible for effector or regulatory reactions. Using models of transplantation immunity and tolerance we show that T cell receptor-mediated recognition of allogeneic or xenogeneic antigens as well as the balance between immunity/tolerance induces distinct cytokine production profiles. The ratio between Th1 and Th2 cytokines efficiently regulates the expression of genes for common enzymes, such as iNOS, arginase, HO-1 and IDO. These enzymes may compete for substrates, such as L-arginine or tryptophan, and the final product of their activity are small molecules (NO, CO) displaying effector or regulatory functions of the immune system. Thus, it is suggested that in spite of the high immunological specificity of transplantation reaction, the ultimate players in regulatory and effector functions could be small and common molecules.

Key words: Immunoregulation; Graft rejection; Tolerance; Th1/Th2 balance; Macrophages; Nitric oxide; Arginase

Core tip: The paper discusses the role of small and common molecules, such are inducible nitric oxide synthase, arginase, heme oxygenase-1 or indoleamine-2,3-dioxygenase, the bioavailability of their substrates (L-arginine, tryptophan, heme) and the cytotoxic and regulatory actions of their small gaseous products (NO, CO), in regulation of transplantation reactions.

Holan V, Krulova M. Common and small molecules as the ultimate regulatory and effector mediators of antigen-specific transplantation reactions. *World J Transplant* 2013; 3(4): 54-61 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/54.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.54

INTRODUCTION

The recognition of graft donor antigens, either by a direct or indirect route, induces an immune response that includes the participation of phenotypically and functionally distinct cell populations. The activity and mutual cooperation of these cells result in the stimulation of effector cytotoxic cells and graft rejection on one side or in the activation of regulatory Tregs (T) cells and regulatory Bregs (B) cells and the induction of transplantation tolerance on the other side. Both effector cytotoxic reactions and transplantation tolerance are strictly haplotype specific.

It is now well recognized that due to the abundance of immunological mechanisms, more different cell populations and a number of different mechanisms are involved in the regulation of the immune reaction and contribute to graft rejection or tolerance induction. Regulatory activity is not restricted to the best characterized $CD4^+CD25^+$ $Foxp3^+$ T cells, as $CD8^+$, $CD8^+CD28^-$, $CD4^-CD8^-$ and NKT cells have been shown to inhibit immune reactions in some models of transplantation tolerance[1-3]. Recently, a regulatory activity, independent of antibody production, has been attributed to a B cell population called B cells[4,5]. These cells inhibit immune reactions, including transplantation immunity[6,7]. Similarly, effector cytotoxic reactions are not confined only to the activity of the originally described cytotoxic $CD8^+$ T cells, but cytotoxic $CD4^+$ T cells, NK cells and especially activated macrophages can kill allogeneic and xenogeneic cells of graft donor origin. Recent data suggest that the specificity and type of transplantation reaction are ensured during the recognition of antigens by the antigen-specific T cell receptor and by the cytokine environment. Different types of transplantation antigens and/or different immunization/tolerization conditions induce distinct patterns of cytokine production (Table 1). The published data indicate that individual cytokines stimulate the expression of functionally different, but in the organism common genes, that are responsible for the generation of small effector molecules representing the ultimate regulatory and effector elements of the immune system. Taking into account the recognized mechanisms of $CD4^+CD25^+$ $Foxp3^+$ T and B cell action and the mechanism of the cytotoxic activity of activated macrophages we suggest that at least some regulatory and effector functions of the immune system are mediated by "common small" molecules that are functionally not confined only to the immune system.

MACROPHAGES AS IMPORTANT EFFECTOR CELLS INVOLVED IN GRAFT REJECTION

For many years, cytotoxic $CD8^+$ T lymphocytes which kill cells of the graft donor haplotype *in vitro*, had been considered as the main effector cells responsible for graft rejection. However, experiments have shown that the depletion of $CD8^+$ T cells does not prevent graft rejection[8]. A more important role in the rejection reaction has been attributed to $CD4^+$ T cells. Elimination of $CD4^+$ T cells results in the prolonged survival of both allografts and xenografts or even in a permanent allograft tolerance[9-11]. An important role in allograft rejection has been attributed to two $CD4^+$ T cell subsets - to proinflammatory Th1 cells producing interleukin (IL)-2 and interferon (IFN)-γ and to Th17 cells producing IL-17[12,13]. In addition to $CD4^+$ T cells, a significant role in graft rejection is played by macrophages, which represent an abundant cell population infiltrating rejected allografts and xenografts[14,15]. The involvement of macrophages in both the recognition and rejection of grafted cells has been described[16,17]. It has been shown in a kidney allograft model that the greatest accumulation of macrophages producing nitric oxide (NO) occurs in those sites in the graft where the greatest degree of damage and the highest occurence of apoptotic graft cells are seen[17].

Macrophages require for their activation a signal from stimulated T cells. It has been demonstrated in various models of allotransplantation that alloantigen-stimulated $CD4^+$ T cells are the main activators of graft infiltrating macrophages and that IFN-γ is the principal cytokine responsible for their activation[18,19]. According to the type of activation signal, two distinct populations of macrophages have been described[20,21]. The so-called classically activated or "killer" macrophages (M1) are activated by IFN-γ (or other Th1 or Th17 cytokines) and produce reactive oxygen species, proinflammatory cytokines and drive an inflammatory/rejection reaction. In contrast, alternatively activated or "healer" macrophages (M2) are stimulated by the Th2 cytokines IL-4 and IL-13 and contribute to debris scavenging, angiogenesis and the wound healing process. Their phenotype and activity can be enhanced by another Th2 cytokine IL-10[22].

Since individual T cell subpopulations differ in their ability to produce different patterns of cytokines and to activate M1 or M2 macrophage subpopulations, the

Table 1 Polarization of cytokine production in response to transplantation antigens

Model	Type of cytokine response[1]
Acute rejection of allograft	Th1 and Th17
MLC to xenoantigens	Th2
Rejection of xenograft	Predominantly Th2
Neonatally induced tolerance of allografts	Th2
Anti-CD4 induced tolerance in adulthood	Th2
Immunosuppressive drug induced tolerance to alloantigens	Th2
Acute graft-versus-host reaction	Th1
Chronic graft-versus-host reaction	Th2
Mucosal tolerance to alloantigens	Th2 (or Th3)

[1]The Th1 type of cytokine response is characterized by the predominant production of interleukin (IL)-2 and interferon γ. For the Th2 type of response, the higher production of IL-4, IL-5, IL-10 and IL-13 is typical. The production of the proinflammatory cytokine IL-17 is characteristic of the Th17 response. The Th3 type of response is characterized by the production of IL-4 and IL-10 and by the high production of the inhibitory cytokine transforming growth factor β.

expression of effector mechanisms of the rejection reaction will depend on the cytokine spectrum at the site of rejection and subsequently on the activity of graft infiltrating macrophages. The classically activated macrophages produce NO as one of the toxic effector molecules involved in graft rejection.

NITRIC OXIDE IN ALLOGRAFT REJECTION

NO is an ubiquious molecule that is toxic for a variety of pathogens and foreign cells. The production of NO is catalyzed in the body by the enzyme nitric oxide synthase (NOS) which occurs in three isoforms: endothelial NOS, neural NOS and inducible NOS (iNOS). Especially iNOS which is expressed in a variety of cells of the immune system and mainly in macrophages, can inducibly produce large quantities of NO. Elevated levels of NO have been detected during the rejection of skin, kidney, heart, liver, lung and corneal allografts[23-25]. The production of NO after allotransplantation correlates with the kinetics of graft rejection and with the fate of the graft[18] and the highest iNOS expression is seen in those sites in an allograft where the highest level of apoptosis of the grafted cells occurs[17]. The observations that the inhibition of NO production by means of specific iNOS inhibitors[18,26,27] or by NO scavenging[28] prevents graft rejection and prolongs allograft survival can be considered as direct evidence for involvement of NO in allograft rejection.

THE RELATIONSHIP BETWEEN INOS/ARGINASE AND NO PRODUCTION

iNOS is expressed in a variety of immunologically active cells, and among them activated macrophages are the main producers of NO. Once induced, iNOS oxidizes L-arginine as a substrate to form NO and citrulline. However, iNOS has to compete for L-arginine with arginase, another intracellular enzyme that utilizes L-arginine. Arginase which converts L-arginine into urea and L-ornithine, is produced in two molecular forms, arginase Ⅰ and arginase Ⅱ. Both isoforms differ in their cellular sublocalization and their tissue distribution. Arginase Ⅰ, the cytosolic isoform, is mainly found in the liver and less so in other tissues, whereas arginase Ⅱ, the mitochondrial isoform, is found predominantly in the kidney, prostate, small intestine, and breast[29]. Significant differences in the tissue expression of arginase isoforms also exist among various species. For example, while mouse macrophages express both isoforms, only arginase Ⅰ was found in rat macrophages[30]. Human arginase Ⅰ can be found among myeloid cells only in granulocytes, and its expression is not modulated by a variety of proinflammatory or anti-inflammatory stimuli[31]. It seems that the genes for both isoforms are regulated differentially and have different kinetics of expression in stimulated cells[30].

Both iNOS and arginase compete for L-arginine as a common substrate and thus affect each other. Biochemical data showed that while Km for L-arginine is in the 2-20 mmol/L range for arginase compared with the 2-20 μmol/L range for various NO synthases, the *V*max of arginase is 1000-fold higher than for NOS[32]. Furthermore, the NOS product hydroxyarginine is an inhibitor of arginase while conversely, polyamines inhibit the NOS enzymes[33]. The amount of NO formed thus depends critically on the bioavailability of the substrate[34]. In other words, the increased formation of arginase decreases the bioavailability of L-arginine for iNOS and thus reduces or even attenuates the production of NO. These biochemical properties are likely to have functional significance since it has been demonstrated that arginase activity in macrophages limits NO production[35,36].

CYTOKINE-INDUCED REGULATION OF THE INOS/ARGINASE RATIO

The expression of both L-arginine utilizing enzymes, iNOS and arginase, is reciprocally regulated by cytokines. While Th1 cytokines stimulate the production of iNOS and rather inhibit the expression of the genes for arginase, Th2 cytokines activate arginase and suppress iNOS formation[35-37]. This dichotomy in the cytokine regulation of the iNOS/arginase ratio is demonstrated in Figure 1. The main cytokine activating iNOS expression and NO production in macrophages is IFN-γ, but other proinflammatory cytokines, such as TNF, IL-1 and IL-17, can also stimulate NO production. The production of arginase is stimulated by Th2 cytokines[35], mainly by IL-4, IL-10, IL-13 and transforming growth factor β. The cytokines that stimulate arginase, suppress the cytotoxic functions of macrophages and inhibit NO production. Thus, it is obvious that the activity of the macrophages participating in an immune response is regulated by the ratio between Th1/Th2 cytokines in the environment. While Th1 cytokines stimulate NO production, the presence or an excess of Th2 cytokines inhibits NO formation through the upregulation of arginase and subsequently by the exhaustion of L-arginine. This differential activation of the enzymes iNOS/arginase is further complicated by the recent discovery of the additional $CD4^+$ proinflammatory T cell subsets Th17 and Th22 which modulate iNOS activity by the production of IL-17 and IL-22[38]. The dichotomy in the upregulation of iNOS or arginase production correlates with the above mentioned M1 or M2 macrophage phenotype[20,21]. M1 macrophages produce iNOS which uses L-arginine as a substrate to produce NO. In contrast, M2 macrophages constitutively produce the enzyme arginase Ⅰ, which sequesters L-arginine from iNOS and results in the production of ornithine and downstream polyamines and L-proline[20].

THE INOS/ARGINASE RATIO DURING GRAFT REJECTION

Macrophages represent an abundant cell population infil-

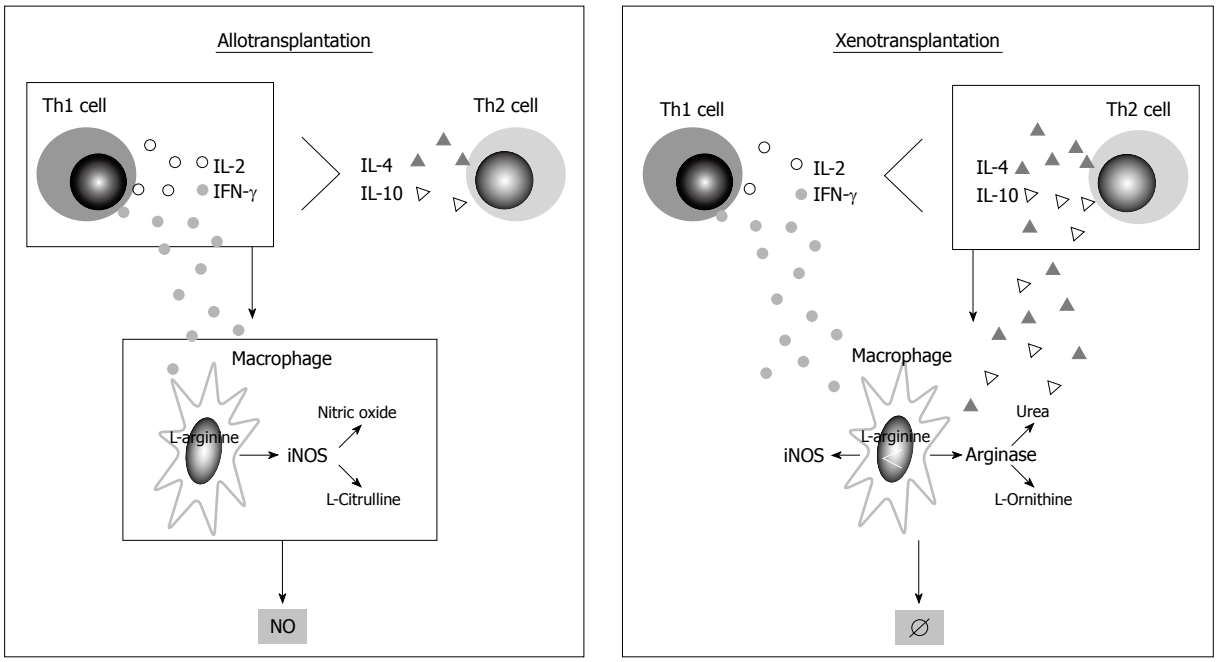

Figure 1 The distinct cytokine production profiles that are induced during allograft or xenograft rejection differentially regulate the expression of the genes for the enzymes inducible nitric oxide synthase and arginase. During allograft rejection, high levels of interleukin (IL)-2 and interferon (IFN)-γ and very low amounts of IL-4 and IL-10 are produced. The proinflammatory cytokine IFN-γ stimulates the expression of the gene for inducible nitric oxide synthase (iNOS), and significant NO generation can be observed in rejected allografts. In contrast, the rejection of xenografts (or allograft tolerance) is associated with the high expression of the genes for the Th2 cytokines IL-4 and IL-10, in addition to the production of Th1 cytokines. Both iNOS and arginase are formed during xenograft rejection. Arginase successfully competes with iNOS for L-arginine as a common substrate. As a consequence, the availability of L-arginine for iNOS becomes limited, and little or no NO generation can be detected in rejected xenografts.

trating rejected allografts and xenografts[39]. Other cell types, such as activated $CD4^+$ or $CD8^+$ T cells, also occur regularly at the site of graft rejection and are a potent source of various cytokines. Therefore, the local cytokine milieu created by various graft-infiltrating T cell subsets regulates the iNOS/arginase ratio and the production of NO by macrophages. Since a strong Th1 cytokine response is regularly observed during allograft rejection[40,41], overexpression of the *iNOS* gene and enhanced NO production can be expected during allograft rejection. Numerous studies have confirmed that increased levels of NO are, in fact, produced during allograft rejection[17-19,42]. Conversely, the higher production of IL-4 and IL-10, *i.e.*, cytokines stimulating arginase, dominates during xenograft rejection or in the state of transplantation tolerance[43-45]. Since arginase utilizes L-arginine with a high affinity, which then becomes less available for iNOS, NO production can be expected to be attenuated. Indeed, we found a lack of NO formation in rejected rat skin xenografts, in spite of abundant *iNOS* gene expression and iNOS protein accumulation in the xenografts[46]. Using selective inhibition of arginase activity with the specific inhibitor N^{ω}-hydroxy-L-arginine, the production of NO in the rejected skin xenografts was restored[47]. Similarly, the production of NO in xenograft explants was restored by adding an excess of L-arginine to the cultures[47]. Furthermore, we demonstrated that the activation of arginase was inhibited or decreased when xenograft recipients were treated with an anti-CD4 mAb, eliminating $CD4^+$ T cells as the principal source of Th2 cytokines after xenotransplantation, or with anti-IL-4 mAb, the antibody neutralizing the main cytokine that activates the expression of the arginase genes. Both of these treatments restored, at least partially, NO production after xenotransplantation. Taken together, these results suggest that the Th1/Th2 ratio during allograft or xenograft rejection regulates NO production through its influence on the iNOS/arginase balance and that $CD4^+$ T cells are the main players regulating this pathway.

GENERAL CONCLUSIONS CONCERNING INOS/ARGINASE REGULATION

The production of NO by graft infiltrating macrophages is effectively regulated by the cytokine milieu at the site of graft rejection. Th1 cytokines which predominate during acute allograft rejection support the development of M1 macrophages, and stimulate iNOS expression and NO production. Conversely, Th2 cytokines which are abundantly produced during the state of allograft tolerance or during the rejection of xenografts, stimulate the activation of M2 macrophages as well as arginase formation and thus cause a decrease in bioavailability of L-arginine for iNOS. As a consequence of this pathway, NO production is attenuated. This regulatory pathway may ensure the absence of NO production as a cytotoxic effector molecule during allograft tolerance. The production of IL-10, a typical Th2 cytokine, is also a main mechanism of Breg-mediated immunosuppression. As

evidence, neutralization of IL-10 abrogates B-cell mediated suppression in a majority of systems[5,48]. The role of B cells in transplantation tolerance has been shown[6,7]. As mentioned above, IL-10 is one of the cytokines that stimulates in macrophages the expression of arginase, which successfully competes with iNOS for the common substrate L-arginin and thus attenuates NO production by iNOS. The absence of NO decreases rejection reaction and supports graft tolerance, Similarly, NO generation is also very low or absent during xenograft rejection which is associated with the elevated production of the Th2 cytokines IL-4 and IL-10. The participation of other cell populations, such as NK cells, eosinophils and cytotoxic $CD8^+$ T cells, which are not so frequent in rejected allografts, or the production of cytotoxic anti-xenograft antibodies can overcome the absence of NO during xenograft rejection.

From a more general point of view, the ability of arginase to inhibit NO generation by competing for L-arginine may have an important physiological significance. High levels of Th2 cytokines and strong arginase activity are regularly induced in the host by different parasite and pathogen infections. It has been demonstrated that the level of host arginase represents a marker of resistence or susceptibility to trypanosome infections[49]. Other studies have suggested that the induction of arginase may represent an evolutionary escape mechanism ensuring the survival of the pathogen[50,51]. The production of arginase by pathogens themselves can represent another mechanism representing a strategy for bacterial survival[52]. Conversely, high NO production during a strong immune response would damage the cells and tissues of the host. In this context, arginase can be considered a protective factor for the host by its ability to lower NO production, which can limit tissue damage or immunosuppression[53]. This may also be the case with the down-regulation of NO production during a strong xenograft reaction, when arginase can limit NO production and thus protect the host tissues from damage by high NO secretion. Therefore, Th2 cytokines stimulating arginase activity in these situations may represent a self-protective mechanism saving the body's own cells from harmful effects of high concentrations of NO.

IMMUNOREGULATORY EFFECTS OF INDOLEAMINE-2,3-DIOXYGENASE

Indoleamine-2,3-dioxygenase (IDO) is an intracellular enzyme that regulates the initial rate-limiting step in tryptophan degradation along the kynurenine pathway[54]. IDO is expressed in various tissues and its expression is induced by IFN-γ and other proinflammatory cytokines[55]. The enzymatic activity of IDO regulates the bioavailability of tryptophan for a cell, and the starvation of tryptophan by its consumption by IDO results in an inhibition of T cell proliferation and activation. In addition, the low molecular weight products of tryptophan metabolism, such as kynurenine derivates and O_2 free radicales, inhibit T cell proliferation and functions[56,57]. The activity of IDO was suggested as a mechanism of the immunosuppressive action of tolerogenic dendritic cells and the suppression mediated by bone marrow-derived mesenchymal stem cells[58,59]. The inhibition of T cell function through tryptophan metabolism and the effects of tryptophan starvation by IDO consumption thus appear as another mechanism involved in the suppression, in a cytokine-dependent manner, of transplantation and other immune reactions[60]. The results indicate that tryptophan is another substrate, similarly as L-arginin, whose concentrations and bioavailability regulate immune reactions and thus can be one of the molecular mechanisms participating in the state of transplantation tolerance.

FINAL CONSIDERATION: ARE "COMMON SMALL" MOLECULES THE ULTIMATE PLAYERS IN THE EFFECTOR AND REGULATORY FUNCTIONS IN THE IMMUNE SYSTEM?

This review suggests that cytokine-induced enzymes, such as NOS, arginase and IDO, and their substrates and products (L-arginine, tryptophan, NO) strongly influence the expression of the cytotoxic effector functions of the immune system. This suggestion is supported by the elucidation of the molecular mechanisms of immunoregulation. An important role in the downregulation of the immune system is played by $CD4^+CD25^+$ T cells, which inhibit the proliferation and cytokine production of other immunocompetent cells[61]. The development and functioning of these T cells are associated with the expression of the forkhead box P3 transcritional factor (Foxp3)[62,63]. It has been suggested that Foxp3 activates the expression of the gene for heme oxygenase-1 (HO-1)[64]. HO-1 catalyzes the degradation of heme and this reaction results in the liberation of equimolar amounts of iron, CO and biliverdin. Since CO has been shown to exert antiproliferative effects[65] and can block IL-2 production[66], this small molecule can be the ultimate effector of T cell-mediated immunosuppression[64]. Indeed, blocking HO-1 in $CD25^+CD4^+$ T cells abrogated their suppressor function[67]. In addition, Oh *et al*[68] demonstrated that the upregulation of HO-1 expression can block the expression of iNOS and NO production, and that CO was responsible for this suppression. Thus, CO produced by the activity of HO-1 expressed in T cells at the site of a tolerated graft can contribute to the suppression of iNOS expression, silencing NO production and to the protection of the graft from the toxic effects of NO.

In summary, the recent data suggest that common molecules, such as NOS, arginase, IDO and HO-1, and their substrates or products, such as L-arginine, tryptophan, NO and CO, are the ultimate players mediating immunoregulatory and effector functions of the immune system.

REFERENCES

1 **Wood KJ**, Bushell A, Hester J. Regulatory immune cells in transplantation. *Nat Rev Immunol* 2012; **12**: 417-430 [PMID: 22627860 DOI: 10.1038/nri3227]
2 **Tang Q**, Bluestone JA, Kang SM. CD4(+)Foxp3(+) regulatory T cell therapy in transplantation. *J Mol Cell Biol* 2012; **4**: 11-21 [PMID: 22170955]
3 **Picarda E**, Anegon I, Guillonneau C. T-cell receptor specificity of CD8(+) Tregs in allotransplantation. *Immunotherapy* 2011; **3**: 35-37 [PMID: 21524168 DOI: 10.2217/imt.11.37]
4 **Mizoguchi A**, Mizoguchi E, Smith RN, Preffer FI, Bhan AK. Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. *J Exp Med* 1997; **186**: 1749-1756 [PMID: 9362534]
5 **Mauri C**, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol* 2012; **30**: 221-241 [PMID: 22224776 DOI: 10.1146/annurev-immunol-020711-074934]
6 **Lee KM**, Kim JI, Stott R, Soohoo J, O'Connor MR, Yeh H, Zhao G, Eliades P, Fox C, Cheng N, Deng S, Markmann JF. Anti-CD45RB/anti-TIM-1-induced tolerance requires regulatory B cells. *Am J Transplant* 2012; **12**: 2072-2078 [PMID: 22494812 DOI: 10.1111/j.1600-6143.2012.04055.x]
7 **Redfield RR**, Rodriguez E, Parsons R, Vivek K, Mustafa MM, Noorchashm H, Naji A. Essential role for B cells in transplantation tolerance. *Curr Opin Immunol* 2011; **23**: 685-691 [PMID: 21982511 DOI: 10.1016/j.coi.2011.07.011]
8 **Rosenberg AS**, Munitz TI, Maniero TG, Singer A. Cellular basis of skin allograft rejection across a class I major histocompatibility barrier in mice depleted of CD8+ T cells in vivo. *J Exp Med* 1991; **173**: 1463-1471 [PMID: 1674524]
9 **Xu BY**, Yang H, Serreze DV, MacIntosh R, Yu W, Wright JR. Rapid destruction of encapsulated islet xenografts by NOD mice is CD4-dependent and facilitated by B-cells: innate immunity and autoimmunity do not play significant roles. *Transplantation* 2005; **80**: 402-409 [PMID: 16082337 DOI: 10.1097/01.tp.0000168107.79769.63]
10 **Pearson TC**, Darby CR, Bushell AR, West LJ, Morris PJ, Wood KJ. The assessment of transplantation tolerance induced by anti-CD4 monoclonal antibody in the murine model. *Transplantation* 1993; **55**: 361-367 [PMID: 8094580 DOI: 10.1097/00007890-199302000-00025]
11 **Yin DP**, Ma LL, Sankary HN, Shen J, Zeng H, Varghese A, Chong AS. Role of CD4+ and CD8+ T cells in the rejection of concordant pancreas xenografts. *Transplantation* 2002; **74**: 1236-1241 [PMID: 12451259 DOI: 10.1097/00007890-200211150-00007]
12 **Chen X**, Zhao S, Tang X, Ge H, Liu P. Neutralization of mouse interleukin-17 bioactivity inhibits corneal allograft rejection. *Mol Vis* 2011; **17**: 2148-2156 [PMID: 21850190]
13 **Heidt S**, Segundo DS, Chadha R, Wood KJ. The impact of Th17 cells on transplant rejection and the induction of tolerance. *Curr Opin Organ Transplant* 2010; **15**: 456-461 [PMID: 20616728 DOI: 10.1097/MOT.0b013e32833b9bfb]
14 **Le Meur Y**, Jose MD, Mu W, Atkins RC, Chadban SJ. Macrophage colony-stimulating factor expression and macrophage accumulation in renal allograft rejection. *Transplantation* 2002; **73**: 1318-1324 [PMID: 11981428 DOI: 10.1097/00007890-200204270-00022]
15 **Fox A**, Mountford J, Braakhuis A, Harrison LC. Innate and adaptive immune responses to nonvascular xenografts: evidence that macrophages are direct effectors of xenograft rejection. *J Immunol* 2001; **166**: 2133-2140 [PMID: 11160265]
16 **Yi S**, Hawthorne WJ, Lehnert AM, Ha H, Wong JK, van Rooijen N, Davey K, Patel AT, Walters SN, Chandra A, O'Connell PJ. T cell-activated macrophages are capable of both recognition and rejection of pancreatic islet xenografts. *J Immunol* 2003; **170**: 2750-2758 [PMID: 12594306]
17 **Szabolcs MJ**, Ravalli S, Minanov O, Sciacca RR, Michler RE, Cannon PJ. Apoptosis and increased expression of inducible nitric oxide synthase in human allograft rejection. *Transplantation* 1998; **65**: 804-812 [PMID: 9539092 DOI: 10.1097/00007890-199803270-00007]
18 **Krulová M**, Zajícová A, Fric J, Holán V. Alloantigen-induced, T-cell-dependent production of nitric oxide by macrophages infiltrating skin allografts in mice. *Transpl Int* 2002; **15**: 108-116 [PMID: 11935167 DOI: 10.1111/j.1432-2277.2002.tb00137.x]
19 **Matuschek A**, Ulbrich M, Timm S, Schneider M, Thomas Germer C, Ulrichs K, Otto C. Analysis of parathyroid graft rejection suggests alloantigen-specific production of nitric oxide by iNOS-positive intragraft macrophages. *Transpl Immunol* 2009; **21**: 183-191 [PMID: 19409993 DOI: 10.1016/j.trim.2009.04.004]
20 **Weisser SB**, McLarren KW, Kuroda E, Sly LM. Generation and characterization of murine alternatively activated macrophages. *Methods Mol Biol* 2013; **946**: 225-239 [PMID: 23179835 DOI: 10.1007/978-1-62703-128-8_14]
21 **Gordon S**, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005; **5**: 953-964 [PMID: 16322748 DOI: 10.1038/nri1733]
22 **Deng B**, Wehling-Henricks M, Villalta SA, Wang Y, Tidball JG. IL-10 triggers changes in macrophage phenotype that promote muscle growth and regeneration. *J Immunol* 2012; **189**: 3669-3680 [PMID: 22933625 DOI: 10.4049/jimmunol.1103180]
23 **Suzuki A**, Kudoh S, Mori K, Takahashi N, Suzuki T. Expression of nitric oxide and inducible nitric oxide synthase in acute renal allograft rejection in the rat. *Int J Urol* 2004; **11**: 837-844 [PMID: 15479287 DOI: 10.1111/j.1442-2042.2004.00910.x]
24 **Lenčová A**, Pokorná K, Zajícová A, Krulová M, Filipec M, Holáň V. Graft survival and cytokine production profile after limbal transplantation in the experimental mouse model. *Transpl Immunol* 2011; **24**: 189-194 [PMID: 21118723 DOI: 10.1016/j.trim.2010.11.005]
25 **Bellos JK**, Perrea DN, Theodoropoulou E, Vlachos I, Papachristodoulou A, Kostakis AI. Clinical correlation of nitric oxide levels with acute rejection in renal transplantation. *Int Urol Nephrol* 2011; **43**: 883-890 [PMID: 20957433 DOI: 10.1007/s11255-010-9858-9]
26 **Worrall NK**, Lazenby WD, Misko TP, Lin TS, Rodi CP, Manning PT, Tilton RG, Williamson JR, Ferguson TB. Modulation of in vivo alloreactivity by inhibition of inducible nitric oxide synthase. *J Exp Med* 1995; **181**: 63-70 [PMID: 7528779 DOI: 10.1084/jem.181.1.63]
27 **Strestíková P**, Plsková J, Filipec M, Farghali H. FK 506 and aminoguanidine suppress iNOS induction in orthotopic corneal allografts and prolong graft survival in mice. *Nitric Oxide* 2003; **9**: 111-117 [PMID: 14623177 DOI: 10.1016/j.niox.2003.08.003]
28 **Roza AM**, Cooper M, Pieper G, Hilton G, Dembny K, Lai CS, Lindholm P, Komorowski R, Felix C, Johnson C, Adams M. NOX 100, a nitric oxide scavenger, enhances cardiac allograft survival and promotes long-term graft acceptance. *Transplantation* 2000; **69**: 227-231 [PMID: 10670631 DOI: 10.1097/00007890-200001270-00006]
29 **Jenkinson CP**, Grody WW, Cederbaum SD. Comparative properties of arginases. *Comp Biochem Physiol B Biochem Mol Biol* 1996; **114**: 107-132 [PMID: 8759304 DOI: 10.1016/0305-0491(95)02138-8]
30 **Salimuddin A**, Gotoh T, Isobe H, Mori M. Regulation of the genes for arginase isoforms and related enzymes in mouse macrophages by lipopolysaccharide. *Am J Physiol* 1999; **277**: E110-E117 [PMID: 10409134]
31 **Munder M**, Mollinedo F, Calafat J, Canchado J, Gil-Lamaignere C, Fuentes JM, Luckner C, Doschko G, Soler G, Eichmann K, Müller FM, Ho AD, Goerner M, Modolell M. Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. *Blood* 2005; **105**: 2549-2556

[PMID: 15546957]
32 **Morris SM**. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr* 2002; **22**: 87-105 [PMID: 12055339 DOI: 10.1146/annurev.nutr.22.110801.140547]
33 **Morris SM Jr**. Regulation of arginine availability and its impact on NO synthesis. In: Ignarro LJ, editor. Nitric Oxide. Biology and Pathobiology. San Diego, CA: Academic Press, 2000: 187-197 [DOI: 10.1016/B978-012370420-7/50012-5]
34 **Morris CR**, Poljakovic M, Lavrisha L, Machado L, Kuypers FA, Morris SM. Decreased arginine bioavailability and increased serum arginase activity in asthma. *Am J Respir Crit Care Med* 2004; **170**: 148-153 [PMID: 15070820 DOI: 10.1164/rccm.200309-1304OC]
35 **Modolell M**, Corraliza IM, Link F, Soler G, Eichmann K. Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. *Eur J Immunol* 1995; **25**: 1101-1104 [PMID: 7537672 DOI: 10.1002/eji.1830250436]
36 **Munder M**, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. *J Immunol* 1998; **160**: 5347-5354 [PMID: 9605134]
37 **Morris SM**, Kepka-Lenhart D, Chen LC. Differential regulation of arginases and inducible nitric oxide synthase in murine macrophage cells. *Am J Physiol* 1998; **275**: E740-E747 [PMID: 9814991]
38 **Mühl H**, Bachmann M, Pfeilschifter J. Inducible NO synthase and antibacterial host defence in times of Th17/Th22/T22 immunity. *Cell Microbiol* 2011; **13**: 340-348 [PMID: 21199257 DOI: 10.1111/j.1462-5822.2010.01559.x]
39 **Wyburn KR**, Jose MD, Wu H, Atkins RC, Chadban SJ. The role of macrophages in allograft rejection. *Transplantation* 2005; **80**: 1641-1647 [PMID: 16378052 DOI: 10.1097/01.tp.0000173903.26886.20]
40 **Wu CJ**, Lovett M, Wong-Lee J, Moeller F, Kitamura M, Goralski TJ, Billingham ME, Starnes VA, Clayberger C. Cytokine gene expression in rejecting cardiac allografts. *Transplantation* 1992; **54**: 326-332 [PMID: 1496544 DOI: 10.1097/00007890-199208000-00024]
41 **Strom TB**, Roy-Chaudhury P, Manfro R, Zheng XX, Nickerson PW, Wood K, Bushell A. The Th1/Th2 paradigm and the allograft response. *Curr Opin Immunol* 1996; **8**: 688-693 [PMID: 8902395 DOI: 10.1016/S0952-7915(96)80087-2]
42 **Holán V**, Krulová M, Zajícová A, Pindjáková J. Nitric oxide as a regulatory and effector molecule in the immune system. *Mol Immunol* 2002; **38**: 989-995 [PMID: 12009578 DOI: 10.1016/S0161-5890(02)00027-5]
43 **Wren SM**, Wang SC, Thai NL, Conrad B, Hoffman RA, Fung JJ, Simmons RL, Ildstad ST. Evidence for early Th 2 T cell predominance in xenoreactivity. *Transplantation* 1993; **56**: 905-911 [PMID: 8212215 DOI: 10.1097/00007890-199310000-00025]
44 **Singh NP**, Guo L, Mhoyan A, Shirwan H. Predominant expression of Th2 cytokines and interferon-gamma in xenogeneic cardiac grafts undergoing acute vascular rejection. *Transplantation* 2003; **75**: 586-590 [PMID: 12640294 DOI: 10.1097/01.TP.0000052594.83318.68]
45 **Holán V**. Transplantation tolerance and cytokines: is suppressor cell activity mediated by Th2 cells? *Folia Biol* (Praha) 1998; **44**: 37-44 [PMID: 10730854]
46 **Holán V**, Pindjáková J, Zajícová A, Krulová M, Zelezná B, Matousek P, Svoboda P. The activity of inducible nitric oxide synthase in rejected skin xenografts is selectively inhibited by a factor produced by grafted cells. *Xenotransplantation* 2005; **12**: 227-234 [PMID: 15807773 DOI: 10.1111/j.1399-3089.2005.00214.x]
47 **Holán V**, Pindjáková J, Krulová M, Neuwirth A, Fric J, Zajícová A. Production of nitric oxide during graft rejection is regulated by the Th1/Th2 balance, the arginase activity, and L-arginine metabolism. *Transplantation* 2006; **81**: 1708-1715 [PMID: 16794538 DOI: 10.1097/01.tp.0000226067.89690.2b]
48 **Klinker MW**, Lundy SK. Multiple mechanisms of immune suppression by B lymphocytes. *Mol Med* 2012; **18**: 123-137 [PMID: 22033729 DOI: 10.2119/molmed.2011.00333]
49 **Duleu S**, Vincendeau P, Courtois P, Semballa S, Lagroye I, Daulouède S, Boucher JL, Wilson KT, Veyret B, Gobert AP. Mouse strain susceptibility to trypanosome infection: an arginase-dependent effect. *J Immunol* 2004; **172**: 6298-6303 [PMID: 15128819]
50 **Noël W**, Hassanzadeh G, Raes G, Namangala B, Daems I, Brys L, Brombacher F, Baetselier PD, Beschin A. Infection stage-dependent modulation of macrophage activation in Trypanosoma congolense-resistant and -susceptible mice. *Infect Immun* 2002; **70**: 6180-6187 [PMID: 12379696 DOI: 10.1128/IAI.70.11.6180-6187.2002]
51 **Iniesta V**, Gómez-Nieto LC, Corraliza I. The inhibition of arginase by N(omega)-hydroxy-l-arginine controls the growth of Leishmania inside macrophages. *J Exp Med* 2001; **193**: 777-784 [PMID: 11257143]
52 **Gobert AP**, McGee DJ, Akhtar M, Mendz GL, Newton JC, Cheng Y, Mobley HL, Wilson KT. Helicobacter pylori arginase inhibits nitric oxide production by eukaryotic cells: a strategy for bacterial survival. *Proc Natl Acad Sci USA* 2001; **98**: 13844-13849 [PMID: 11717441]
53 **Millar AE**, Sternberg J, McSharry C, Wei XQ, Liew FY, Turner CM. T-Cell responses during Trypanosoma brucei infections in mice deficient in inducible nitric oxide synthase. *Infect Immun* 1999; **67**: 3334-3338 [PMID: 10377110]
54 **Mellor AL**, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004; **4**: 762-774 [PMID: 15459668 DOI: 10.1038/nri1457]
55 **Däubener W**, MacKenzie CR. IFN-gamma activated indoleamine 2,3-dioxygenase activity in human cells is an antiparasitic and an antibacterial effector mechanism. *Adv Exp Med Biol* 1999; **467**: 517-524 [PMID: 10721095 DOI: 10.1007/978-1-4615-4709-9_64]
56 **Frumento G**, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J Exp Med* 2002; **196**: 459-468 [PMID: 12186838]
57 **Munn DH**, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol* 2013; **34**: 137-143 [PMID: 23103127 DOI: 10.1016/j.it.2012.10.001]
58 **Munn DH**, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slingluff CL, Mellor AL. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* 2002; **297**: 1867-1870 [PMID: 12228717 DOI: 10.1126/science.1073514]
59 **Meisel R**, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; **103**: 4619-4621 [PMID: 15001472]
60 **Curti A**, Trabanelli S, Salvestrini V, Baccarani M, Lemoli RM. The role of indoleamine 2,3-dioxygenase in the induction of immune tolerance: focus on hematology. *Blood* 2009; **113**: 2394-2401 [PMID: 19023117]
61 **Wood KJ**, Sakaguchi S. Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003; **3**: 199-210 [PMID: 12658268 DOI: 10.1038/nri1027]
62 **Hori S**, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; **299**: 1057-1061 [PMID: 12522256 DOI: 10.1126/science.1079490]
63 **Fontenot JD**, Rudensky AY. Molecular aspects of regulatory T cell development. *Semin Immunol* 2004; **16**: 73-80 [PMID: 15036230 DOI: 10.1016/j.smim.2003.12.002]

64 **Choi BM**, Pae HO, Jeong YR, Kim YM, Chung HT. Critical role of heme oxygenase-1 in Foxp3-mediated immune suppression. *Biochem Biophys Res Commun* 2005; **327**: 1066-1071 [PMID: 15652505 DOI: 10.1016/j.bbrc.2004.12.106]

65 **Ryter SW**, Otterbein LE, Morse D, Choi AM. Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance. *Mol Cell Biochem* 2002; **234-235**: 249-263 [PMID: 12162441 DOI: 10.1023/A:1015957026924]

66 **Pae HO**, Oh GS, Choi BM, Chae SC, Kim YM, Chung KR, Chung HT. Carbon monoxide produced by heme oxygenase-1 suppresses T cell proliferation via inhibition of IL-2 production. *J Immunol* 2004; **172**: 4744-4751 [PMID: 15067050]

67 **Brusko TM**, Wasserfall CH, Agarwal A, Kapturczak MH, Atkinson MA. An integral role for heme oxygenase-1 and carbon monoxide in maintaining peripheral tolerance by CD4+CD25+ regulatory T cells. *J Immunol* 2005; **174**: 5181-5186 [PMID: 15843512]

68 **Oh GS**, Pae HO, Choi BM, Chae SC, Lee HS, Ryu DG, Chung HT. 3-Hydroxyanthranilic acid, one of metabolites of tryptophan via indoleamine 2,3-dioxygenase pathway, suppresses inducible nitric oxide synthase expression by enhancing heme oxygenase-1 expression. *Biochem Biophys Res Commun* 2004; **320**: 1156-1162 [PMID: 15249210 DOI: 10.1016/j.bbrc.2004.06.061]

P- Reviewer: Song SH **S- Editor**: Wen LL **L- Editor**: A
E- Editor: Zheng XM

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.62

World J Transplant 2013 December 24; 3(4): 62-67
ISSN 2220-3230 (online)

EDITORIAL

Clinical and ethical considerations of massively parallel sequencing in transplantation science?

Andreas Scherer

Andreas Scherer, Spheromics, 81100 Kontiolahti, Finland
Author contributions: Scherer A solely contributed to this paper.
Correspondence to: Andreas Scherer, PhD, APM, CEO, Spheromics, Lehmustie 16, 81100 Kontiolahti, Finland. andreas. scherer@spheromics.com
Telephone: +358-44-3629559 Fax: +358-44-3629559
Received: June 25, 2013 Revised: September 12, 2013
Accepted: October 11, 2013
Published online: December 24, 2013

Abstract

Massively parallel sequencing (MPS), alias next-generation sequencing, is making its way from research laboratories into applied sciences and clinics. MPS is a framework of experimental procedures which offer possibilities for genome research and genetics which could only be dreamed of until around 2005 when these technologies became available. Sequencing of a transcriptome, exome, even entire genomes is now possible within a time frame and precision that we could only hope for 10 years ago. Linking other experimental procedures with MPS enables researchers to study secondary DNA modifications across the entire genome, and protein binding sites, to name a few applications. How the advancements of sequencing technologies can contribute to transplantation science is subject of this discussion: immediate applications are in graft matching *via* human leucocyte antigen sequencing, as part of systems biology approaches which shed light on gene expression processes during immune response, as biomarkers of graft rejection, and to explore changes of microbiomes as a result of transplantation. Of considerable importance is the socio-ethical aspect of data ownership, privacy, informed consent, and result report to the study participant. While the technology is advancing rapidly, legislation is lagging behind due to the globalisation of data requisition, banking and sharing.

Key words: Sequencing; Diagnosis; Ethics; Consent; Data management

Core tip: Despite the great excitement about the opportunities that massively parallel sequencing (MPS) bears for the promotion of, *e.g.*, transplant science, personalized medicine faces the challenge to guarantee privacy of data and findings. Here, some applications of MPS in transplant science are mentioned, and concerns and challenges in data analysis and management are discussed.

Scherer A. Clinical and ethical considerations of massively parallel sequencing in transplantation science?. *World J Transplant* 2013; 3(4): 62-67 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/62.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.62

MASSIVELY PARALLEL SEQUENCING

Massively parallel sequencing (MPS) is an alias of the probably more popular term next-generation sequencing (NGS). In this article the term NGS will be avoided for the simple reason that "N" is a relative term in matter of time. If Sanger's and Maxam and Gilbert's ground breaking inventions of DNA sequencing in 1974 is counted as the first generation, then the automation of Sanger's method could be considered the next or second generation. Further, the next step which in 2005 lead to the development of machines which were able to sequence millions of fragments of DNA simultaneously would certainly have to be called the next-next-generation or third generation[1]. And the next generation of technological improvement is on its way: the 4th generation of sequencing methodology will utilize entire strands of DNA without the need of fragmentation, and will become cheaper, and more precise, and simpler to handle bioinformatically. In

order to avoid counting generations of technological advancement, the term MPS, or MPS for short, seems more applicable and will be used herein.

A variety of technical approaches to MPS exist, all of them have been reviewed in detail (for an excellent overview please see, *e.g.*[2]. Briefly, the general principle relies on (1) the fragmentation of DNA/RNA, optionally followed by fragment size selection; (2) amplification of the fragments; and (3) sequencing of the fragments. Currently, the length of those sequences can be up to 800 bp, depending on the vendor. As the fragmentation step generates random breakpoints in the DNA backbone, so will the sequenced fragments in Step 3 be at a random position of the DNA or RNA. This is where the individual small pieces of sequence information will have to be bioinformatically stitched together, "assembled", being a challenge to which there are plenty of approaches with slightly different quality, depending on the analysis pipeline of choice.

The applications of MPS are overwhelming and offer never seen before opportunities to study genomes, exomes, transcriptomes, and chromosomal rearrangements and secondary modifications like methylation of DNA and alkylation of RNA. Through its unbiased template-free approach, it is now also possible to sequence DNA and RNA of novel species in *de novo* assembly analyses and thus accelerate discovery of, *e.g.*, ontological relationships[3], and even discover novel RNA species[4]. Input amounts in the low ng-range for some MPS applications make it possible to study biological samples in a detail which could not have been envisioned before[5]. MPS has found its way to the analysis of single eukaryotic cells or even cell-free DNA in blood samples, *e.g.* for non-invasive prenatal diagnosis[6].

PERSONALIZED TREATMENT

Following transplantation, drug treatment must be carefully adjusted to prevent rejection. Drug metabolism is influenced by a large variety of factors such as age, gender, disease, dose, drug-drug interaction, and metabolic competence. Differences in the genotype (polymorphisms) can be linked with altered drug metabolism in transplant patients. Tacrolimus for example is primarily metabolized *via* the CYP450 enzymes. Non-expressors of CYP3A5 metabolize the drug slower than others, hence requiring lower doses than normal expressors[7]. Similarly, in conjunction with age as variant, polymorphisms in the transporter ABCB1 can determine the bioavailability of cyclosporine and mycophenolate mofetil[8]. Another example which illustrates the importance of studying genetic polymorphisms to optimize personal treatment is the occurrence of hypertension after transplantation. In genome-wide association studies polymorphisms have been identified in a number of genes affecting hypertension (*e.g.*[9]). For overviews of the field please see the recent reviews of D'Alessandro *et al*[10] and of Kurzawski *et al*[11]. As these examples and other studies, which cannot be discussed here for space limitation, show, the individual landscapes of polymorphisms in patients need to be assessed to optimize treatment efficacy. Sequencing of genes with standard methods is time-consuming and can deliver ambiguous. MPS technology can be used to study exomes of patients through targeted sequencing of candidate genes and determine polymorphisms which may affect treatment. However, MPS does not always deliver unambiguous results either due to sequence coverage differences and DNA sequence specifics such as guanine-cytosine (GC) content or homopolymers which cannot always be resolved by current MPS technologies alone. At times, one may need to verify the results by alternative technologies to obtain further sequence information.

HUMAN LEUCOCYTE ANTIGEN MATCHING

Alleles of the human leucocyte antigen (*HLA*) genes are commonly used for organ and bone marrow matching prior to transplantation. Humans vary widely in the composition of antigens arising from alleles of those six *HLA* genes (*A*, *B*, *C*, *DR*, *DQ*, *DP*). Detection of foreign HLA antigens by the host can lead to strong antibody mediated reactions, thus they can be considered important mediators of immune response. During the graft selection process, it is therefore essential to detect donor-host HLA mismatches, a process commonly performed by Sanger sequencing of the HLA locus. While Sanger sequencing certainly has its merits, technical limitations such as relatively high sequence inaccuracy resulting in sequence ambiguity due to highly polymorphic DNA regions, and limited sequence coverage in a single experiment (only a small number of exons is sequenced systematically, and some important polymorphisms may be located outside the sequenced regions) may make another round of experimental verification necessary in many cases. With ever decreasing costs, MPS has the potential to deliver high-quality sequence data which cover a large proportion of the entire HLA locus[12,13].

IMMUNE SYSTEM

MPS can be applied to many aspects of biological research in the transplantation arena. Exon arrays and RNA sequencing was applied to address the question whether alternative splicing takes place during immune response post-transplant. The group of Grigoryev *et al*[14] purified human CD2(+) T or CD19(+) B cells, activated them to model early post-transplant immune events and continued to sample from those cell pools over time. Indeed they were able to show that these two cell populations not only regulate gene expression following *in vitro* stimuli, but also regulate exon usage to generate alternative panels of transcripts which may contribute to the biological pattern of immune response. MPS now permits devising experiments which aim at studying the methylation status of DNA of T cells and B cells before, during and after immune response, *e.g.*, graft rejection. Methylation of promoter regions plays an important role in gene

regulation[15]. Changes of the methylation status of genes during immune response during and after treatment hence may give clues about how the expression of genes is regulated, for example in combination with DNA-protein motif discovery. For excellent overviews of MPS methods for the investigation of epigenetic modifications of DNA and RNA, please see[16,17].

METAGENOMICS

16S rRNA pyrosequencing is a variety of MPS used to selectively sequence the highly variable 16S rRNA regions of bacterial genomes, thus providing qualitative and quantitative genus and species information of bacteria present in a sample[18]. The group of Diaz *et al*[19] used 16S rRNA pyrosequencing to study the bacteriome of the human oral cavity after transplantation. They demonstrated a shift in the composition of the microbiome of the oral cavity during immunosuppression following transplantation[19]. The authors speculate that immunosuppression may create an environment in the oral cavity which could be more permissive for opportunistic pathogens.

A number of groups have focused on characterizing the microbiome of alveolar fluid in relation to lung transplantation. For instance, Borewizc *et al*[20] have applied 16S rRNA pyrosequencing to study the human lung microbiome after lung transplantation. The authors compiled sequencing data from 12 bronchoalveolar lavage fluid samples from four patients over three time points, two additional samples from healthy, non-transplanted individuals served as controls. Interestingly they found that the microbial diversity increased after transplantation, and that the dominating phyla after transplantation were different from those in healthy lungs. The authors suggest to follow those results under the aspect of the bronchiolitis obliterans syndrome, which is a marker of chronic lung transplant rejection[21].

DIAGNOSIS

In 2012, Wen *et al*[22] demonstrated that the number of circulating endothelial cells (CECs) increased in whole blood of renal transplant patients undergoing acute rejection, acute tubular necrosis, and chronic allograft nephropathy, when compared to control samples. CEC count decreased after immunosuppressive therapy. The authors attributed the increased CEC count to injury of vessel endothelium in conjunction with endarteritis, and conclude that monitoring CEC numbers can be used as minimally invasive tool to diagnose or prognose poor short-term outcome of renal allografts. Technically, it is not farfetched to design scenarios in which MPS technologies could be applied to monitor the number of CECs in whole blood samples. Whole genome sequencing would not be necessary; an exon-capture set specific for exons of endothelial genes would suffice for qualitative measurement of CECs. On the quantitative side read numbers would have to be normalized against a set of stably expressed genes, identification of which can be challenging, as seen in the microarray arena.

Certainly, similar to other conventional approaches such as microarrays, MPS can be used to develop biomarkers of rejection or tolerance. Despite striving to identify the best matching grafts for hosts, the best matches are not always tolerated. The reason for tolerance, or lack thereof, may be found not within coding region of the HLA, but possibly in surrounding (introns, promoters) or even distant genomic areas. With ever decreasing costs of MPS it will soon be possible to sequence not only exons or exomes in a larger scale than possible or affordable today, but entire genomes. As is the case in other research disciplines it will be necessary to gather genomic sequence information from a sufficient number of individuals to draw significant conclusions. This is the case for mutation analysis [*e.g.*, single nucleotide polymorphisms (SNP)], as well as the analysis of secondary modifications such as methylation when certain biological conditions are compared. Research will see a steady growth of available sequence information which will contribute to discovery and qualification of biomarkers and elucidation of biological processes for the benefit of patients.

BIOINFORMATICS CHALLENGES

There are now many MPS-approaches to sequencing DNA, which will continue to reduce speed and cost of sequencing. When in 2000, still in the pre-MPS era, the drafts of a human genome sequence were published, one would not have thought that already 13 years on, the cost for this undertaking would come down from around $3B to around $5-10K, and the sequencing and analysis time would shrink from 10 years "for a rough working draft" to around 3-4 wk on the average for a complete version. However, decreased sequence raw data generation time and costs mean huge challenges for IT in terms of data storage and transfer of the huge raw data files which can be in the TB range per run, and for bioinformatics data analysis capacities, including quality control, alignment, assembly, annotation, and statistical analysis. No longer is the data generation process the experimental bottleneck, but the analytical side of things. In fact, as Sboner *et al*[23] phrase it, there is an "unpredictable amount of extra 'human' time" which is required for the identification of the best analysis pipelines, software installation, *etc.* Like in the early days of microarrays experts argue about the approaches to data processing. This leads to an amount of approaches which can be even overwhelming for bioinformaticians themselves (if they would admit it): What is the most precise, fastest, aligner, assembler, normalization method, algorithm to identify SNPs, statistics for differentially expressed genes, differentially methylated sites, *etc.*? Some methods are listed in[2]. Evaluating which analysis pipeline suits best to which problem and to which IT environment is challenging and time consuming. The final step, the interpretation of the results, is yet another "unknown" time factor which can rarely be done automatically, but requires human intervention. In the end one needs to understand that sequencing

cannot in every case provide an immediate answer to all scientific questions. Just like in all other comparative experiments which we have become familiar with over the years, the first step in experiments involving MPS is sampling. Sampling means that individuals are selected which represent the entire group of individuals we are interested in, a process which can be attempted in a variety of statistical approaches of experimental study design, such as randomization, blocking, and randomization[24]. Many sequencing applications do not omit the need for biological replicates, a cost-factor which needs to be considered in the planning phase. Certainly this is true for transcriptome analysis, differential methylation analysis, but also for genome-wide association studies (GWAS). The latter will benefit dramatically from the increased precision and availability of whole genome information in the near future, contributing to the growing number of lead mutations in diseases (for an overview of GWAS studies, www.genome.gov/GWAstudies). MPS will allow the discovery of rare variants where commonly used SNP arrays will have to fail. Certainly, there are settings where one sample will suffice. These are occasions in which individual information about a genome is investigated, *e.g.,* in cancer-genomics or in rare diseases. This brings our discussion to the aspect of personalized medicine and MPS.

DATA MANAGEMENT AND PRIVACY

Decreasing costs and increasing availability of resources will make MPS a tool for medical research and clinical care. However, routine genome sequencing for patient care brings along important socio-ethical and legal ramifications which are heavily discussed. Crucial concerns arise around patient information to obtain informed consent, data protection and patient privacy protection, data ownership, third-party use, use of incidental findings, and how such (incidental) findings are disclosed to the patient, to name a few[25-27]. On the other hand, sequencing data can be used for a whole range of scientific and clinical applications, becoming accessible *via* databases across nations. Sequence data can be used *e.g.* for trait analysis, phylogenetic testing, and expression analysis, bringing along a wide range of possible findings which is difficult to estimate at the time of sampling. Hence, to obtain informed consent from a patient the extent of consent has to be fairly thorough, which may cause frustration and possibly unwillingness to consent, additionally posing risks of study bias due to social background. McGuire *et al*[28] proposed a tiered consent process with three levels, from intended release of data information on multiple gene loci, to single gene loci, to releasing no data. Sample donors would have to be educated about the risks and benefits of the foreseen use of their data. Data access would have to be restricted according to the intended use at the beginning of the study. Reconsideration of study purposes may enforce re-consenting.

If genomic information is released though, is it possible to fully protect the privacy of sequencing data? Already in the pre-MPS era of 2004, Malin and his team showed that it was possible to link genomic data to named individuals in publicly available records by leveraging unique features in patient-location visit patterns[29]. With the growth of genome sequence databases it should be possible to identify individuals based on their DNA sequence (*e.g.* SNP pattern), provided a template is present. In 2004, Lin *et al*[30] published it was possible to de-identify a person by interrogating just 75 SNPs, not many when taking into consideration that SNP databases of human genomes contain hundreds of thousands per genome. Not only the patient's but also the relatives' privacy is affected, but may be affected. This has large implications not only on research, but even more importantly on health care systems and national databases. The goal of the Health Insurance Portability and Accountability Act of 1996 is to protect genomic data as personal health information (http://www.hhs.gov/ocr/privacy).

The extent of result disclosure poses another issue. How much does a patient need to learn about the results, especially incidental findings, which were not part of the original study. What are results and who is interpreting them? As Sharp pointed out in a detailed discussion[31], the amount of data and potential findings with all their false positives and negatives, is equally overwhelming for the practitioner as it will be for the study participant. Many mutations may be harmless, and a result-interpretation may again be interpreted as a result by a study participant[31].

These are only a few critical concerns that have to be addressed urgently. The scientific community needs to ensure that the legal and ethical framework which makes social discrimination based on genetic information impossible is appropriate for the developing technology. International databases and cloud computing impose the necessity of international legislation which puts the patient rights first. By ensuring privacy protection, study participation has a chance to be beneficial for the individual, not a potential risk for social exclusion.

OUTLOOK

Over the next years prices per sequenced nucleotide will continue to fall, sequencing machines will become smaller, cheaper and easier to use, eventually making genomic sequencing a standard tool in research and clinics. Despite growing databases, MPS data interpretation will remain a challenge. The legal and ethical frameworks for using MPS data need to be defined on an international level, granting respect to sample-providing individuals as well as the research goals of scientists and clinicians. International consortia need to address the possibility that the current speed of genome research may outrun the pace of legal regulation, and impose adjustments.

REFERENCES

1 **Margulies M**, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z,

Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 2005; **437**: 376-380 [PMID: 16056220 DOI: 10.1038/nature03959]

2 **Xuan J**, Yu Y, Qing T, Guo L, Shi L. Next-generation sequencing in the clinic: Promises and challenges. *Cancer Lett* 2013; **340**: 284-295 [PMID: 23174106 DOI: 10.1016/j.canlet.2012.11.025]

3 **Wang M**, Ye Y, Tang H. A de Bruijn graph approach to the quantification of closely-related genomes in a microbial community. *J Comput Biol* 2012; **19**: 814-825 [PMID: 22697249 DOI: 10.1089/cmb.2012.0058]

4 **Martens-Uzunova ES**, Olvedy M, Jenster G. Beyond microRNA - Novel RNAs derived from small non-coding RNA and their implication in cancer. *Cancer Lett* 2013; **340**: 201-211 [PMID: 23376637 DOI: 10.1016/j.canlet.2012.11.058]

5 **Sengupta S**, Ruotti V, Bolin J, Elwell A, Hernandez A, Thomson J, Stewart R. Highly consistent, fully representative mRNA-Seq libraries from ten nanograms of total RNA. *Biotechniques* 2010; **49**: 898-904 [PMID: 21143212 DOI: 10.2144/000113556]

6 **Nepomnyashchaya YN**, Artemov AV, Roumiantsev SA, Roumyantsev AG, Zhavoronkov A. Non-invasive prenatal diagnostics of aneuploidy using next-generation DNA sequencing technologies, and clinical considerations. *Clin Chem Lab Med* 2013; **51**: 1141-1154 [PMID: 23023923 DOI: 10.1515/cclm-2012-0281]

7 **MacPhee IA**, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW. The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. *Am J Transplant* 2004; **4**: 914-919 [PMID: 15147425 DOI: 10.1111/j.1600-6143.2004.00435.x]

8 **Fanta S**, Niemi M, Jönsson S, Karlsson MO, Holmberg C, Neuvonen PJ, Hoppu K, Backman JT. Pharmacogenetics of cyclosporine in children suggests an age-dependent influence of ABCB1 polymorphisms. *Pharmacogenet Genomics* 2008; **18**: 77-90 [PMID: 18192894 DOI: 10.1097/FPC.0b013e3282f3ef72]

9 **Levy D**, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; **41**: 677-687 [PMID: 19430479 DOI: 10.1038/ng.384]

10 **D'Alessandro LCA**, Mital S. Pediatric tranplantion: opportunities for pharmacogenomics and genomics. *PERS MED* 2013; **10**: 397-404

11 **Kurzawski M**, Droździk M. Pharmacogenetics in solid organ transplantation: genes involved in mechanism of action and pharmacokinetics of immunosuppressive drugs. *Pharmacogenomics* 2013; **14**: 1099-1118 [PMID: 23837483 DOI: 10.2217/pgs.13.89]

12 **De Santis D**, Dinauer D, Duke J, Erlich HA, Holcomb CL, Lind C, Mackiewicz K, Monos D, Moudgil A, Norman P, Parham P, Sasson A, Allcock RJ. 16(th) IHIW : review of HLA typing by NGS. *Int J Immunogenet* 2013; **40**: 72-76 [PMID: 23302098 DOI: 10.1111/iji.12024]

13 **Erlich RL**, Jia X, Anderson S, Banks E, Gao X, Carrington M, Gupta N, DePristo MA, Henn MR, Lennon NJ, de Bakker PI. Next-generation sequencing for HLA typing of class I loci. *BMC Genomics* 2011; **12**: 42 [PMID: 21244689 DOI: 10.1186/1471-2164-12-42]

14 **Grigoryev YA**, Kurian SM, Nakorchevskiy AA, Burke JP, Campbell D, Head SR, Deng J, Kantor AB, Yates JR, Salomon DR. Genome-wide analysis of immune activation in human T and B cells reveals distinct classes of alternatively spliced genes. *PLoS One* 2009; **4**: e7906 [PMID: 19936255 DOI: 10.1371/journal.pone.0007906]

15 **Sibbritt T**, Patel HR, Preiss T. Mapping and significance of the mRNA methylome. *Wiley Interdiscip Rev RNA* 2013; **4**: 397-422 [PMID: 23681756 DOI: 10.1002/wrna.1166]

16 **Ku CS**, Naidoo N, Wu M, Soong R. Studying the epigenome using next generation sequencing. *J Med Genet* 2011; **48**: 721-730 [PMID: 21825079 DOI: 10.1136/jmedgenet-2011-100242]

17 **Rodriguez BA**, Frankhouser D, Murphy M, Trimarchi M, Tam HH, Curfman J, Huang R, Chan MW, Lai HC, Parikh D, Ball B, Schwind S, Blum W, Marcucci G, Yan P, Bundschuh R. Methods for high-throughput MethylCap-Seq data analysis. *BMC Genomics* 2012; **13** Suppl 6: S14 [PMID: 23134780 DOI: 10.1186/1471-2164-13-S6-S14]

18 **Woese CR**, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci U S A* 1977; **74**: 5088-5090 [PMID: 270744]

19 **Diaz PI**, Hong BY, Frias-Lopez J, Dupuy AK, Angeloni M, Abusleme L, Terzi E, Ioannidou E, Strausbaugh LD, Dongari-Bagtzoglou A. Transplantation-associated long-term immunosuppression promotes oral colonization by potentially opportunistic pathogens without impacting other members of the salivary bacteriome. *Clin Vaccine Immunol* 2013; **20**: 920-930 [PMID: 23616410 DOI: 10.1128/CVI.00734-12]

20 **Borewicz K**, Pragman AA, Kim HB, Hertz M, Wendt C, Isaacson RE. Longitudinal analysis of the lung microbiome in lung transplantation. *FEMS Microbiol Lett* 2013; **339**: 57-65 [PMID: 23173619 DOI: 10.1111/1574-6968.12053]

21 **Jaramillo A**, Fernández FG, Kuo EY, Trulock EP, Patterson GA, Mohanakumar T. Immune mechanisms in the pathogenesis of bronchiolitis obliterans syndrome after lung transplantation. *Pediatr Transplant* 2005; **9**: 84-93 [PMID: 15667618 DOI: 10.1111/j.1399-3046.2004.00270.x]

22 **Wen J**, Chen J, Ji SM, Cheng D, Liu ZH. Evaluation of vascular lesions using circulating endothelial cells in renal transplant patients. *Clin Transplant* 2012; **26**: E344-E350 [PMID: 22515202 DOI: 10.1111/j.1399-0012.2012.01620.x]

23 **Sboner A**, Mu XJ, Greenbaum D, Auerbach RK, Gerstein MB. The real cost of sequencing: higher than you think! *Genome Biol* 2011; **12**: 125 [PMID: 21867570 DOI: 10.1186/gb-2011-12-8-125]

24 **Grass P**. Experimental Design. In: Scherer A, editor Batch Effects and Noise in Microarray Experiments: Sources and Solutions: Wiley and Sons, 2009: 19-31

25 **Greenbaum D**, Sboner A, Mu XJ, Gerstein M. Genomics and privacy: implications of the new reality of closed data for the field. *PLoS Comput Biol* 2011; **7**: e1002278 [PMID: 22144881 DOI: 10.1371/journal.pcbi.1002278]

26 **Mathieu G**, Groisman IJ, Godard B. Next generation sequencing in psychiatric research: what study participants need to know about research findings. *Int J Neuropsychopharmacol* 2013; **16**: 2119-2127 [PMID: 23725748 DOI: 10.1017/S1461145713000527]

27 **Ong FS**, Grody WW, Deignan JL. Privacy and data management in the era of massively parallel next-generation sequencing. *Expert Rev Mol Diagn* 2011; **11**: 457-459 [PMID: 21707452 DOI: 10.1586/ERM.11.34]

28 **McGuire AL**, Gibbs RA. Genetics. No longer de-identified. *Science* 2006; **312**: 370-371 [PMID: 16627725 DOI: 10.1126/science.1125339]

29 **Malin B**, Sweeney L. How (not) to protect genomic data privacy in a distributed network: using trail re-identification to evaluate and design anonymity protection systems. *J Biomed Inform* 2004; **37**: 179-192 [PMID: 15196482 DOI: 10.1016/j.jbi.2004.04.005]
30 **Lin Z**, Owen AB, Altman RB. Genetics. Genomic research and human subject privacy. *Science* 2004; **305**: 183 [PMID: 15247459 DOI: 10.1126/science.1095019]
31 **Sharp RR**. Downsizing genomic medicine: approaching the ethical complexity of whole-genome sequencing by starting small. *Genet Med* 2011; **13**: 191-194 [PMID: 21311340 DOI: 10.1097/GIM.0b013e31820f603f]

P- Reviewers: Kelesidis T, Schuurman HJ **S- Editor**: Cui XM
L- Editor: A **E- Editor:** Yan JL

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.68

World J Transplant 2013 December 24; 3(4): 68-77
ISSN 2220-3230 (online)

REVIEW

Novel immunosuppressive agents in kidney transplantation

Karen L Hardinger, Daniel C Brennan

Karen L Hardinger, Department of Pharmacy Practice and Administration, University of Missouri-Kansas City, Kansas City, MO 64108, United States
Daniel C Brennan, Department of Medicine, Washington University School of Medicine in St. Louis, St. Louis, MO 63110, United States
Author contributions: All authors have made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published.
Correspondence to: Karen L Hardinger, PharmD, BCPS, Clinical Associate Professor of Pharmacy Practice, Department of Pharmacy Practice and Administration, University of Missouri-Kansas City, 2464 Charlotte Street, Rm 2241, Kansas City, MO 64108, United States. hardingerk@umkc.edu
Telephone: +1-816-2769023 Fax: +1-816-2764751
Received: June 28, 2013 Revised: August 26, 2013
Accepted: October 16, 2013
Published online: December 24, 2013

Abstract

Excellent outcomes have been achieved in the field of renal transplantation. A significant reduction in acute rejection has been attained at many renal transplant centers using contemporary immunosuppressive, consisting of an induction agent, a calcineurin inhibitor, an antiproliferative agent plus or minus a corticosteroid. Despite improvements with these regimens, chronic allograft injury and adverse events still persist. The perfect immunosuppressive regimen would limit or eliminate calcineurin inhibitors and/or corticosteroid toxicity while providing enhanced allograft outcomes. Potential improvements to the calcineurin inhibitor class include a prolonged release tacrolimus formulation and voclosporin, a cyclosporine analog. Belatacept has shown promise as an agent to replace calcineurin inhibitors. A novel, fully-human anti-CD40 monoclonal antibody, ASKP1240, is currently enrolling patients in phase 2 trials with calcineurin minimization and avoidance regimens. Another future goal of transplant immunosuppression is effective and safe treatment of allograft rejection. Novel treatments for antibody mediated rejection include bortezomib and eculizumab. Several investigational agents are no longer being pursed in transplantation including the induction agents, efalizumab and alefacept, and maintenance agents, sotrastaurin and tofacitinib. The purpose of this review is to consolidate the published evidence of the effectiveness and safety of investigational immunosuppressive agents in renal transplant recipients.

Key words: Review; Immunosuppression; Investigational agents; Renal/kidney transplant

Core tip: Many new agents are being studied that may improve outcomes after renal transplantation. Potential improvements to the calcineurin inhibitor class include a recently Food and Drug Administration approved, prolonged release tacrolimus formulation and voclosporin, a cyclosporine analog. A novel, fully-human anti-CD40 monoclonal antibody, ASKP1240, is currently enrolling patients in phase 2 trials with calcineurin minimization and avoidance regimens. Novel treatments for antibody mediated rejection include bortezomib and eculizumab.

Hardinger KL, Brennan DC. Novel immunosuppressive agents in kidney transplantation. *World J Transplant* 2013; 3(4): 68-77 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/68.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.68

INTRODUCTION

Although significant advances in renal transplant immunosuppression have occurred in the past decades, a vital need to improve long-term survival remains. Currently, immunologic causes of early graft failure have become rare, yet late graft loss has remained virtually unchanged. One of the most common causes for graft loss is chronic

Table 1 Summary of investigational and available agents

Generic	Brand	FDA indication	Company
Alefacept[1,2]	Amevive	Treatment of moderate-to-severe chronic plaque psoriasis in adults who are candidates for systemic therapy or phototherapy	Astellas
Alemtuzumab[2]	Campath	Treatment of B-CLL	Berlex Laboratories
ASKP1240		Not FDA approved	Astellas
Azathioprine	Imuran	Adjunctive therapy in prevention of rejection of kidney transplants; management of active rheumatoid arthritis	Generic manufacturers
Basiliximab	Simulect	Prevention of acute rejection in kidney transplantation	Novartis
Belatacept	Nulojix	Prevention of acute rejection in renal transplant recipients	Bristol-Myers-Squibb
Bortezomib	Velcade	Treatment of multiple myeloma; treatment of relapsed or refractory mantle cell lymphoma	Millenium Pharmaceuticals
Cyclosporine	Neoral	Prevention of acute rejection in renal transplant recipients	Novartis
Eculizumab	Soliris	Treatment of PNH to reduce hemolysis and aHUS	Alexion Pharmaceuticals
Efalizumab[1,2]	Raptiva	Management of moderate to severe chronic plaque psoriasis in adults	Genentech
Everolimus	Afinitor, Zortress	Treatment of advanced renal cell cancer (Afinitor®); treatment of subependymal giant cell astrocytom associated with tuberous sclerosis (Afinitor®); treatment of advanced, metastatic or unresectable pancreatic neuroendocrine tumors (Afinitor®); prophylaxis of organ rejection in patients at low-moderate immunologic risk receiving renal transplants (Zortress®)	Novartis
Mycophenolate Mofetil	Cellcept	Prophylaxis of organ rejection concomitantly with cyclosporine and corticosteroids in patients receiving allogeneic renal cardiac, or hepatic transplants	Genentech
Mycophenolate Sodium	Myfortic	Prophylaxis of organ rejection concomitantly with cyclosporine and corticosteroids in patients receiving allogeneic renal transplantation	Novartis
Horse or Rabbit anti-thymocyte Globulin	Atgam or Thymoglobulin	Treatment of corticosteroid resistant rejection in kidney transplantation	Pfizer/Sanofi
Rituximab	Rituxan	Treatment of CD20-positive non-Hodgkin's lymphomas ; Treatment of moderately- to severely-active rheumatoid arthritis in adult patients with inadequate response to one or more TNF antagonists; Treatment of Wegener's granulomatosis; Treatment of microscopic polyangiitis	Genentech
Sirolimus	Rapamune	Prevention of acute rejection in renal transplant recipients	Pfizer
Sotrastaurin, AEB-0711		Not FDA approved	Novartis
Tacrolimus	Prograf	Prevention of acute rejection in renal transplant recipients	Astellas
Tacrolimus Prolonged Release	Astragraf XL	Preventing organ rejection in kidney transplant recipients, as combination therapy with mycophenolate mofetil and corticosteroids, with or without tasiliximab induction	Astellas
Tolfacitinib[1]	Xeljanz	Treatment of moderate to severe rheumatoid arthritis	Pfizer
Voclosporin		Not FDA approved	Isotechnika Pharma

[1]No longer being investigated for transplantation; [2]Withdrawn from United States Market. FDA: Food and Drug Administration; B-CLL: B-cell chronic lymphocytic leukemia; PNH: Paroxysmal nocturnal hemoglobinuria; aHUS: Atypical hemolytic uremic syndrome.

allograft nephropathy. Additionally, significant drug improvements in transplantation have come with the expense of side effects. Many of these adverse events, including new onset diabetes after transplant, dyslipidemia, and hypertension, may contribute to cardiovascular related deaths after transplantation. The ideal immunosuppressive regimen would improve long-term outcomes while minimizing exposure to drug toxicity and infection.

Induction agents are typically antibodies (anti-thymocyte globulins) or interleukin 2 receptor antagonists (basiliximab). Another induction agent, alemtuzumab has been removed from the United States market, but is still available through a special manufacturer program. The five drug classes that currently comprise maintenance regimens may include calcineurin inhibitors (cyclosporine and tacrolimus), mTOR inhibitors (sirolimus and everolimus), antiproliferative agents (azathioprine and mycophenolic acid), costimulation blockers (belatacept) and corticosteroids (Table 1). KDIGO Clinical Practice Guidelines suggest that first-line agents should include basiliximab induction for low-risk patients and an anti-thymocyte globulin for high-risk patients in conjunction with maintenance immunosuppression including tacrolimus and mycophenolate[1]. Potential improvements to the calcineurin inhibitor class include a prolonged release tacrolimus formulation and voclosporin, a cyclosporine analog (Table 2). A novel, anti CD-40 molecule has completed phase 1 studies.

Another area for improvement is treatment of humoral rejection. Historically, treatment has been difficult and not well studied. Humoral rejection is typically treated with intravenous immunoglobulin, rituximab and plasmapheresis. Investigational treatments for antibody mediated rejection that will be discussed include bortezomib and eculizumab.

In the past years, several clinical trials have been unsuccessful and therefore many agents are no longer being pursued for transplantation. These agents include two inductions agents, efalizumab and alefacept, and two maintenance agents, sotrastaurin (a protein kinase C in-

Table 2 Non-Food and Drug Administration approved/ investigational agents and their mechanism

Drug name	Mechanism of action
Induction	
Efalizumab[1]	Humanized antibody, CD11a/LFA-1
Alefacept[1]	Costimulation inhibitor, CD2 LFA3
Maintenance	
Voclosporin, ISA247	Calcineurin inhibitor
Sotrastaurin, AEB0711	Protein kinase C inhibitor
Tofacitinib, CP-6905501	JAK 3 inhibitor
ASKP1240	Anti-CD40 monoclonal antibody
Treatment of Antibody Medicated Rejection	
Bortezomib	Proteasome inhibitor
Eculizumab	Monoclonal antibody, C5 complement protein

[1]No longer being investigated for transplantation.

hibitor) and tofacitinib (a JAK 3 inhibitor). This review article will update a previously published article found in this journal[2] on the effectiveness and safety of novel immunosuppressive agents.

PREVENTATIVE AGENTS

Alternatives to currently available calcineurin inhibitors

Calcineurin inhibitors have revolutionized post-transplantation immunosuppressive regimens by significantly lowering acute rejections rates. Yet, long-term use of these drugs has been associated with the development of chronic allograft nephropathy and adverse events. New immunosuppressive agents that eliminate these issues are needed. Prolonged release tacrolimus (Astragraf XL®, Astellas) has been approved for use in various European countries, Canada and the United States (in July 2013). The expectation is that the products once daily, rather than twice daily, dosing will improve adherence in transplant recipients. Large, randomized, phase 3 studies have compared prolonged release-tacrolimus compared to tacrolimus with similar efficacy and safety outcomes[3,4]. Of note, tacrolimus levels may be slightly lower with prolonged release tacrolimus compared to twice daily tacrolimus patients[5-7], although serum creatinine, creatinine clearance and estimated glomerular filtration rate were very similar. Prolonged release tacrolimus has a non-inferior efficacy profile with convenient daily dosing which is expected to improve patient compliance. Drug cost may influence the widespread use of this product as generic tacrolimus formulations are now available.

A novel calcineurin inhibitor, voclosporin (ISA 247, Isotechnika Pharma, Inc.) is being investigated in solid organ transplant, uveitis, and psoriasis[8-11]. Animal studies demonstrated that voclosporin, a cyclosporine analogue, had a higher affinity and greater *in-vivo* potency[12,13]. PROMISE, a phase 2b trial of low risk renal transplant recipients with immediate allograft function (n = 334) compared low (0.4 mg/kg), medium (0.6 mg/kg) and high (0.8 mg/kg) dose voclosporin to tacrolimus (0.05 mg/kg), in combination with a standard immunosuppressive regimen (anti-CD25 antibody, mycophenolate mofetil, and corticosteroids). Rejection rates were non-inferior to tacrolimus (11%, 9%, 2 %, and 6% respectively) and renal function was clinically similar (69-72 mL/min) at 6 mo after transplantation[8]. The incidence of new onset diabetes after transplantation was significantly lower in the low dose voclosporin group (1.6% *vs* 16.4% tacrolimus), but not in the medium (5.7%) and high dose (17.7%) arms[8]. The major limitation of this trial was that only low risk patients were studied. Low to medium dose voclosporin may provide adequate immunosuppression with a lower incidence of new onset diabetes after transplantation. A large, phase 3 (n = 598) trial is planned for 2013.

Recently, pharmacokinetic data of voclosporin was presented at the American Transplant Congress[14-17]. Researchers have learned that voclosporin should be given on an empty stomach and that dosage adjustment may be needed in severe renal failure (< 30 mL/min) and mild to moderate hepatic impairment[14-16]. Optimal trough concentrations should be targeted between 35-60 ng/mL[17].

Belatacept (Nulojix®, Bristol Myers Squibb) is a second generation co-stimulation blocker (CD80 antagonism) that received Food and Drug Administration (FDA) approval for use in kidney transplantation in June of 2011. Belatacept is contraindicated in patients that are Ebstein-Barr virus seronegative, because of high incidence of post-transplant lymphoproliferative seen in clinical trials[19-22]. Belatacept is administered as a well-tolerated intravenous infusion over 30 min. The recommended dosing is 10 mg/kg administered, prior to transplantation, on day 5, and at the end of weeks 2, 4, 8, and 12, then 5 mg/kg every 4 wk (plus or minus 3 d). The chronic intravenous administration could prove beneficial in increasing patient compliance with less frequent (monthly) infusions. In contrast, it may be perceived as a barrier to patients without social support that cannot readily access an infusion center. Administration and drug costs may also influence prescribing patterns and patient compliance.

Belatacept is the first immunosuppressive to demonstrate a renal benefit over a calcineurin inhibitor based regimen[18-22]. One limitation of the early belatacept trials (BENEFIT and BENEFIT-EXT) was that cyclosporine, a less contemporary immunosuppressive, was utilized[19-22]. In a phase 2, 1 year randomized study, belatacept/mycophenolate mofetil, belatacept/sirolimus and tacrolimus/mycophenolate mofetil, in combination with rabbit antithymocyte globulin and without corticosteroids were compared (n = 89)[23]. Acute rejection was highest in the belatacept/mycophenolate mofetil arm, graft loss was lowest in the tacrolimus/mycophenolate arm, and renal function was improved in the belatacept arms.

As an alternative to *de novo* immunosuppression, a conversion trial recently tested the hypothesis that belatacept-based regimens may provide a treatment option in patients already being treated with calcineurin-based maintenance immunosuppression[24]. Patients with stable

graft function (calculated glomerular filtration rate between 35-75 mL/min) were randomized to either switch to belatacept (n =84) or continue calcineurin inhibitor treatment (n = 89). Despite a higher acute rejection rate in the belatacept group, the relative renal benefit of belatacept was observed in patients switched from either cyclosporine (+7.8 mL/min) or tacrolimus (+8.9 mL/min), and was observed regardless of baseline renal function. Patient survival, graft survival and the overall safety profile was similar between groups.

The impact of belatacept on long-term cardiovascular profiles is yet to be determined. An analysis of the pooled data from the BENEFIT AND BENEFIT-EXT trials showed lower blood pressures, lower non HDL cholesterol, lower triglycerides and less new onset diabetes mellitus after transplanation in the belatacept-treated patients versus the cyclosporine treated patients[25]. Yet, in a post-hoc analysis in patients with pre-existing diabetes from the BENEFIT and BENEFIT-EXT, 12 mo patient survival, graft survival, and renal function were similar between belatacept and cyclosporine treated patients[26]. Further trials are needed to explore the long-term outcomes, the impact of Epstein-Barr virus on post-transplant lymphoproliferative disease, and chronic allograft nephropathy. These trials should include contemporary immunosuppressive regimens.

A fully human anti-CD40 monoclonal antibody, ASKP1240 (Astellas®), has shown promise in phase 1 studies[27-29]. The first human, phase 1 study of healthy subjects (n = 12) demonstrated that the antibody was safe and well-tolerated[28]. Subsequently, a phase 1b trial, was performed in *de novo* kidney transplant recipients that received a single intravenous dose of 50 mg (n = 10), 100 mg (n = 9), 200 mg (n = 10), 500 mg (n = 9) or placebo (n = 8), no induction and standard maintenance immunosuppression per each center's protocol[26]. ASKP1240 exhibited non-linear pharmacokinetics and was well tolerated at all doses. Acute rejection occurred in 3 patients in the 50 mg arm, 3 patients in the 500 mg arm and 1 patient in the placebo arm. The incidence of infection was not dose dependent. A phase 2 trial will compare the efficacy of ASKP1240 with calcineurin avoidance (basiliximab induction, ASKP1240, mycophenolate mofetil, and steroids) to the standard of care immunosuppressive regimen (basiliximab induction + tacrolimus + mycophenolate mofetil + steroids). In addition, the study will compare the efficacy of calcineurin inhibitor minimization-mycophenolate mofetil avoidance (basiliximab induction, ASKP1240, tacrolimus and steroids) to the standard of care immunosuppressive regimen.

TREATMENT OF ANTIBODY MEDIATED REJECTION

Antibody mediated rejection is an important cause of acute and chronic graft failure. Acute and chronic antibody mediated rejections are difficult to treat, because they are typically less responsive to conventional anti-rejection therapy. Treatment regimens for acute antibody mediated rejection may include one or more of the following: plasmapheresis, intravenous immunoglobulin (IVIG), and rituximab[30-38], although these regimens are not well-studied. A recent meta-analysis of over 10000 citations on treatment of antibody-mediated rejection concluded that data describing these treatments are of low or very low quality[34]. The first, prospective, randomized study comparing these strategies (plasmapheresis/IVIG/rituximab *vs* IVIG alone) demonstrated improved graft survival in the combination group[38]. Little guidance is given by the KDIGO Clinical Practice Guidelines, they suggest treating antibody-mediated acute rejection with one or more of the following alternatives with or without corticosteroids: plasma exchange; intravenous immunoglobulin; anti-CD20 antibody; lymphocyte-depleting antibody (Grade 2C Recommendation)[1]. Two investigational treatments for antibody mediated rejection include bortezomib and eculizumab.

Bortezomib (Velcade®, Millenium Pharmaceuticals) has been used for treatment of acute antibody mediated rejection, although it is approved for multiple myeloma in the United States (2010). It inhibits the degradation of cell-cycle regulatory proteins resulting in cell-cycle death *via* apoptosis. Bortezomib is metabolized via the cytochrome P450 system, a major substrate of 2C19 and 3A4 and inhibitor of 2C19, and therefore several drug interactions may occur including ketoconazole, clopidogrel, and grapefruit juice. Adverse events associated with bortezomib may include neutropenia, thrombocytopenia, nausea, vomiting, diarrhea, constipation (up to 50%), peripheral neuropathy (up to 30%), hypotension, QT prolongation, heart failure, pneumonitis and pneumonia. One case series of 52 transplant patients treated for antibody mediated rejection or desensitization reported bortezomib associated toxicity to be low, most commonly reported as manageable anemia or peripheral neuropathy[39]. Dosing of bortezomib is 1.3 mg/m^2 on days 1, 4, 8, 11. No adjustments are necessary for renal impairment, but the dosage should be reduced by one-half for moderate to severe hepatic impairment.

Case series have reported the use of bortezomib to remove HLA antibodies in live-donor transplant recipients with HLA alloantibodies[40,41] and to treat antibody and cell-mediated acute rejection[42-51]. Few comparative trials have been performed. One German, historical control study of 10 bortezomib-treated patients (4 doses of 1.3 mg/m^2) *vs* 9 rituximab-treated patients (one fixed dose of 500 mg) with antibody mediated rejection showed improved survival in the bortezomib treated group with an 18 mo graft survival of 60% *vs* 11% in the rituximab group[52]. All patients received plasmapheresis and intravenous immune globulin (30 g). Randomized trials are needed to determine the influence of bortezomib on antibody removal.

Eculizumab (Soliris®, Alexion Pharmaceuticals) is a humanized monoclonal IgG antibody that binds to complement protein C5 and blocks the activation of terminal complement. It is FDA approved for paroxysmal

Table 3 Clinical trials

Agent	Identifier	Study name	Start date
ASKP1240	NCT01780844	A Study to Assess the Efficacy and Safety of ASKP1240 in de Novo Kidney Transplant Recipients	February 2013
Voclosporin	NCT01586845	Safety and Efficacy Study of Voclosporin and Tacrolimus in Transplantation	March 2013
Prolonged Release Tacrolimus	NCT01294020	Study to Compare the Pharmacokinetics of Tacrolimus in Stable Pediatric Allograft Recipients Converted From Prograf® to Advagraf®	May 2011
Bortezomib	NCT01873157	Bortezomib in Late Antibody-mediated Kidney Transplant Rejection (BORTEJECT)	October 2013
	NCT01349595	Impact of Proteasome Inhibition on Anti-Donor HLA Antibody Production After Kidney Transplantation	December 2011
	NCT01842074	Desensitization With Bortezomib Before a Living Kidney Donation (VELDON)	January 2013
	NCT01502267	Desensitization Protocol for Highly Sensitized Patients on the Waiting List for Kidney Transplant	January 2010
	NCT00722722	The Impact of Velcade on Antibody Secreting Cells in Sensitized Renal Allograft Candidates	June 2008
Eculizumab	NCT01349595	Impact of Proteasome Inhibition on Anti-Donor HLA Antibody Production After Kidney Transplantation	December 2011
	NCT01327573	Eculizumab Therapy for Chronic Complement-Mediated Injury in Kidney Transplantation	March 2011
	NCT01095887	Eculizumab Added to Conventional Treatment in the Prevention of Antibody-mediated Rejection in ABO Blood Group Incompatible Living Donor Kidney Transplantation (ABOi)	March 2010
	NCT01403389	A Study of the Activity of Eculizumab for Prevention of Delayed Graft Function In Deceased Donor Kidney Transplant	August 2011
	NCT01567085	Safety and Efficacy Of Eculizumab In The Prevention Of Antibody Mediated Rejection (AMR) In Sensitized Recipients Of A Kidney Transplant From A Deceased Donor	May 2012
	NCT01106027	Dosing Regimen of Eculizumab Added to Conventional Treatment in Positive Crossmatch Deceased Donor Kidney Transplant	March 2010
	NCT01399593	Safety and Efficacy of Eculizumab to Prevent AMR in Living Donor Kidney Transplant Recipients Requiring Desensitization	September 2011

nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. The most common side effects are headache, nausea, fatigue, back pain, cough and nasopharyngitis. Fatal immune hemolytic anemia following allogeneic stem cell transplantation has been reported[53]. Vaccination with meningococcal vaccine at least 2 wk prior to initiation of treatment with continued long-term prophylaxis is recommended. Eculizumab should not be used in patients with serious infections.

Case studies in renal transplant recipients have reported successful treatment of atypical hemolytic uremic syndrome and thrombotic microangiopathy with eculizumab[54-66]. Eculizumab has also been successful in reducing antibodies in a highly sensitized patient with positive crossmatches prior to live donor transplant[67] and in prevention of antibody mediated rejection in a case series of patients with donor specific antibodies and positive flow cytometry cross-matches (n = 4)[68]. In a larger case-control study, patients with donor specific antibodies who received pre-transplant plasmapheresis and post-transplant eculizumab were compared to historical controls[69]. At a median follow up of 12 mo for the eculizumab group, antibody mediated rejection occurred in 7.7 % (2/26) in the eculizumab group compared to 41% (21/51) in the control group ($P < 0.01$). One-year protocol biopsy revealed transplant glomerulopathy in 6.7% (1/15) eculizumab-treated recipients and in 35.7% (15/42) of control patients ($P = 0.044$). Eculizumab use has also been described in an ABO-incompatible deceased-donor kidney and pancreas transplant with a severe antibody-mediated rejection[70,71].

Eculizumab 600 mg weekly for six doses with plasmapheresis has also been successful in reversing refractory, early (mean time 6.5 d), acute antibody mediated rejection in four transplant recipients[72]. Mean follow up time is 6.4 ± 5.7 mo, and while antibodies persisted in the majority of the patients, the allografts are functioning and infectious complications have not occurred. Successful use of eculizumab has also been reported in two patients with antibody mediated rejection associated with thrombotic microangiopathy[73] and three patients with resistant antibody mediated rejection[74,75].

Despite the small sample size and lack of randomized controls, these studies are encouraging, and although larger studies and long-term follow-up are needed, bortezomib and eculizumab may play a major role in acute antibody mediated therapy in the future. Their role in transplant desensitization may be better elucidated as more clinical data and well-designed clinical trials become available. Current and future trials of bortezomib and eculizumab are listed in Table 3[76].

AGENTS NO LONGER BEING INVESTIGATED

Efalizumab (Raptiva®, Genentech) works an immunosuppressant by binding to the CD11a subunit of lymphocyte function-associated antigen 1 (LFA-1) and inhibiting white blood cell migration. This once weekly intramus-

cular injection was indicated for the treatment of chronic moderate-to-severe plaque psoriasis, but has been associated with an increased risk for progressive multifocal leukoencephalopathy (PML) and was withdrawn from the market in April of 2009[77]. Likewise, clinical trials in renal transplant recipients have not been successful due to higher rates of lymphoproliferative disease[78].

Alefacept (Amevive®, Astellas Pharmaceuticals) is a CD2-LFA3 co-stimulation inhibitor[79,80], was an intramuscular injection indicated for treatment of moderate-to-severe chronic plague psoriasis. Alefacept was voluntarily withdrawn from the market by Astellas Pharmaceuticals in December of 2011 due to "business needs"[81]. Prior to the discontinuation, alefacept was being developed for use in conjunction with tacrolimus, mycophenolate mofetil and steroids in renal transplantation. In a phase 2, *de novo* study of adult kidney transplant patients alefacept (*vs* placebo) resulted in similar survival and rejection rates, however the incidence of malignancy was higher in the alefacept arm[82].

Sotrastaurin (AEB071, Novartis), a protein kinase inhibitor, initially proved to have a good tolerability profile with few adverse effects[83]. Sotrastaurin development has been halted due high rejection rates (up to 40%) in *de novo* transplant recipients despite promising results with renal function and a low toxicity profile[83-87].

Tofacitinib (Xeljanz®, Tofacitinib CP-690550, Pfizer Inc.), is a kinase inhibitor with immunosuppressant properties that was FDA approved for moderate to severe rheumatoid arthritis in November of 2012. Tofacitinib is a small molecule agent which exhibits selective inhibition for the JAKs, thus inactivating the JAK/STAT dependent IL-2 induced T-cell proliferation.

Tofacitinib was being studied as a drug to be used in place of calcineurin inhibitors along with other antimetabolite agents in two phase 2 clinical trials. In a small, initial, clinical study on *de novo* kidney allograft recipients comparing a tofacitinib regimen at 15 mg twice daily (CP15) and 30 mg twice daily (CP30) with tacrolimus, researchers reported the 6-mo biopsy-proven acute rejection rates to be 1 of 20, 4 of 20 and 1 of 21 for CP15, CP30 and tacrolimus groups respectively and concluded the 15 mg *bid* regimen to be similar to the tacrolimus regimen[88]. All patients received interleukin-2 receptor antagonist induction, mycophenolic acid and corticosteroids. In a subsequent, larger phase-2 trial (n = 331), a standard cyclosporine regimen was compared with a 15 mg twice daily regimen of tofacitinib which is subsequently switched to 10 mg twice daily after 3 mo (less-intensive) and another 15 mg twice daily regimen of tofacitinib which is switched to 10 mg twice daily after 6 mo (more-intensive)[89]. The biopsy proven acute rejection at 6 mo with the low-dose group (11%) was lower than the more-intensity or cyclosporine groups (7% and 9%, respectively). In terms of glomerular filtration rate at 12 mo, the tofacitinib groups (less-intensity: 65 mL/min and more-intensity: 65 mL/min) showed a significant difference in preservation of renal function compared to the cyclosporine group (54 mL/min). In this study, there was a lower incidence of chronic allograft nephropathy in the more intense and less intense groups (25% and 24% respectively) compared to the cyclosporine group (48%).

The smaller clinical study reported a high incidence of BK virus in the CP30 group (4/20) and a higher 6 mo rate of CMV disease (4/20) compared to CP15 and tacrolimus (2/20 and 0/20 respectively)[88]. Some other common abnormalities noted with this agent were trends towards higher lipid elevations, anemia and neutropenia during the first 6 mo of the treatment when the mycophenolate mofetil dose was high. In the larger, phase 2 trial, there were fewer cases of new-onset diabetes in the more-intense and less-intense groups (9.9% and 9.3% respectively) compared to cyclosporine (20.8%)[89]. The rate of serious infections, BK virus nephritis, post-transplant lymphoproliferative disorder and CMV disease was higher in the tofacitinib groups. The overall findings of the phase 2 studies suggest that tofacitinib is effective in preventing acute rejection and chronic allograft nephropathy, although this was achieved at the expense of hematological toxicity and over-immunosuppression when used in combination with mycophenolate mofetil. Although research has shown that safety may be improved by concentration-controlled dosing[90], tofacitinib development has been discontinued.

CONCLUSION

Induction agents are typically antibodies (anti-thymocyte globulins) or interleukin 2 receptor antagonists (basiliximab). Alemtuzumab has been removed from the United States market, but is available through the manufacturer through a special program. Many questions remain surrounding the use of potent induction agents including whether or not the use is associated with infection and malignancy, if the use is cost-effective, and if there is a true graft survival benefit. Due to poor clinical outcomes, induction investigational agents including, efalizumab and alefacept, are no longer being studied. Maintenance immunosuppressives may show some promise with future novel agents. Prolonged release tacrolimus provides once daily dosing of this product and hopefully will simplify a complex post-transplant immunosuppressive regimen. It is unknown if the perceived benefits will outweigh the cost of this product. Voclosporin, a cyclosporine analog, has not shown superior efficacy outcomes, but perhaps improvement in the safety profile (namely new-onset diabetes after transplant) will secure its place in transplant immunotherapy as the phase 3 trials are underway. ASKP, an anti-CD40 antibody, has successfully completed phase 1 studies and phase 2 trials are ongoing. Although belatacept has shown promise, two other investigational maintenance agents, sotrastaurin and tolfacitinib, will not be studied further in transplantation.

Treatment regimens for acute humoral rejection may include one or more of the following: plasmapheresis, intravenous immunoglobulin, and rituximab. Investigations of bortezomib and eculizumab have been hindered by small, non-randomized trials. Although results are

encouraging, larger studies and long-term follow-up is ongoing.

At this point in time, there are very few immunosuppressants in clinical trials. Although some investigational agents have shown promise, tailoring available agents may need to be the short-term focus for transplant recipients. Hopefully, modifying exist regimens and approval of investigational agents will satisfy the ultimate goal of transplantation to improve long-term survival without toxicity or infection.

REFERENCES

1 **Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group**. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9** Suppl 3: S1-155 [PMID: 19845597 DOI: 10.1111/j.1600-6143.2009.02834.x]

2 **Kalluri HV**, Hardinger KL. The current state of renal transplant immunosuppression: present and future. *World J Transplant* 2012; **2**: 51-68 [DOI: 10.5500/wjt.v2.i4.51]

3 **Silva HT**, Yang HC, Abouljoud M, Kuo PC, Wisemandle K, Bhattacharya P, Dhadda S, Holman J, Fitzsimmons W, First MR. One-year results with extended-release tacrolimus/MMF, tacrolimus/MMF and cyclosporine/MMF in de novo kidney transplant recipients. *Am J Transplant* 2007; **7**: 595-608 [PMID: 17217442 DOI: 10.1111/j.1600-6143.2007.01661.x]

4 **Krämer BK**, Charpentier B, Bäckman L, Silva HT, Mondragon-Ramirez G, Cassuto-Viguier E, Mourad G, Sola R, Rigotti P, Mirete JO. Tacrolimus once daily (ADVAGRAF) versus twice daily (PROGRAF) in de novo renal transplantation: a randomized phase III study. *Am J Transplant* 2010; **10**: 2632-2643 [PMID: 20840480 DOI: 10.1111/j.1600-6143.2010.03256.x]

5 **Jelassi ML**, Lefeuvre S, Karras A, Moulonguet L, Billaud EM. Therapeutic drug monitoring in de novo kidney transplant receiving the modified-release once-daily tacrolimus. *Transplant Proc* 2011; **43**: 491-494 [PMID: 21440742 DOI: 10.1016/j.transproceed.2011.01.043]

6 **Hougardy JM**, Broeders N, Kianda M, Massart A, Madhoun P, Le Moine A, Hoang AD, Mikhalski D, Wissing KM, Abramowicz D. Conversion from Prograf to Advagraf among kidney transplant recipients results in sustained decrease in tacrolimus exposure. *Transplantation* 2011; **91**: 566-569 [PMID: 21192316 DOI: 10.1097/TP.0b013e3182098ff0]

7 **Kitada H**, Okabe Y, Nishiki T, Miura Y, Kurihara K, Terasaka S, Kawanami S, Tuchimoto A, Masutani K, Tanaka M. One-year follow-up of treatment with once-daily tacrolimus in de novo renal transplant. *Exp Clin Transplant* 2012; **10**: 561-567 [PMID: 23082898 DOI: 10.6002/ect.2012.0087]

8 **Busque S**, Cantarovich M, Mulgaonkar S, Gaston R, Gaber AO, Mayo PR, Ling S, Huizinga RB, Meier-Kriesche HU. The PROMISE study: a phase 2b multicenter study of voclosporin (ISA247) versus tacrolimus in de novo kidney transplantation. *Am J Transplant* 2011; **11**: 2675-2684 [PMID: 21943027 DOI: 10.1111/j.1600-6143.2011.03763.x]

9 **Naidoo P**, Rambiritch V. Voclosporin (ISA247) for plaque psoriasis. *Lancet* 2008; **372**: 888-889; author reply 889 [PMID: 18790303 DOI: 10.1016/S0140-6736(08)61391-4]

10 **Papp K**, Bissonnette R, Rosoph L, Wasel N, Lynde CW, Searles G, Shear NH, Huizinga RB, Maksymowych WP. Efficacy of ISA247 in plaque psoriasis: a randomised, multicentre, double-blind, placebo-controlled phase III study. *Lancet* 2008; **371**: 1337-1342 [PMID: 18424323 DOI: 10.1016/S0140-6736(08)60593-0]

11 **Bissonnette R**, Papp K, Poulin Y, Lauzon G, Aspeslet L, Huizinga R, Mayo P, Foster RT, Yatscoff RW, Maksymowych WP. A randomized, multicenter, double-blind, placebo-controlled phase 2 trial of ISA247 in patients with chronic plaque psoriasis. *J Am Acad Dermatol* 2006; **54**: 472-478 [PMID: 16488299 DOI: 10.1016/j.jaad.2005.10.061]

12 **Kuglstatter A**, Mueller F, Kusznir E, Gsell B, Stihle M, Thoma R, Benz J, Aspeslet L, Freitag D, Hennig M. Structural basis for the cyclophilin A binding affinity and immunosuppressive potency of E-ISA247 (voclosporin). *Acta Crystallogr D Biol Crystallogr* 2011; **67**: 119-123 [PMID: 21245533 DOI: 10.1107/S0907444910051905]

13 **Gregory CR**, Kyles AE, Bernsteen L, Wagner GS, Tarantal AF, Christe KL, Brignolo L, Spinner A, Griffey SM, Paniagua RT, Hubble RW, Borie DC, Morris RE. Compared with cyclosporine, ISATX247 significantly prolongs renal-allograft survival in a nonhuman primate model. *Transplantation* 2004; **78**: 681-685 [PMID: 15371668 DOI: 10.1097/01.TP.0000131950.75697.71]

14 **Aspeslet L**, Freitag D, Huizinga R, Mayo P, Ling S, Foster R. The pharmacokinetics of voclosporin (VCS) in renal impairment. *Am J Transpl* 2013; **13** Suppl 5: abstr B1020

15 **Aspeslet L**, Freitag D, Huizinga R, Mayo P, Ling S, Foster R. The effects of hepatic impairment on single and multiple oral dose pharmacokinetics of voclosporin (VCS). *Am J Transpl* 2013; **13** Suppl 5: abstr B1033

16 **Aspeslet L**, Freitag D, Huizinga R, Mayo P, Ling S, Foster R. The effects of high fat breakfast, low fat breakfast or fasting on the absorption of voclosporin (VCS). *Am J Transpl* 2013; **13** Suppl 5: abstr B1032

17 **Alloway R**, Bloom R, Gaston R, Goggins M, Mayo P, Busque S. A comparison of voclosporin and tacrolimus 1 year outcomes as predicted by PKPD in the PROMISE trial. *Am J Transpl* 2013; **13** Suppl 5: abstr B1018

18 **Vincenti F**, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, Lang P, Grinyo J, Halloran PF, Solez K, Hagerty D, Levy E, Zhou W, Natarajan K, Charpentier B. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**: 770-781 [PMID: 16120857 DOI: 10.1056/NEJMoa050085]

19 **Vincenti F**, Charpentier B, Vanrenterghem Y, Rostaing L, Bresnahan B, Darji P, Massari P, Mondragon-Ramirez GA, Agarwal M, Di Russo G, Lin CS, Garg P, Larsen CP. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant* 2010; **10**: 535-546 [PMID: 20415897 DOI: 10.1111/j.1600-6143.2009.03005.x]

20 **Larsen CP**, Grinyó J, Medina-Pestana J, Vanrenterghem Y, Vincenti F, Breshahan B, Campistol JM, Florman S, Rial Mdel C, Kamar N, Block A, Di Russo G, Lin CS, Garg P, Charpentier B. Belatacept-based regimens versus a cyclosporine A-based regimen in kidney transplant recipients: 2-year results from the BENEFIT and BENEFIT-EXT studies. *Transplantation* 2010; **90**: 1528-1535 [PMID: 21076381 DOI: 10.1097/TP.0b013e3181ff87cd]

21 **Durrbach A**, Pestana JM, Pearson T, Vincenti F, Garcia VD, Campistol J, Rial Mdel C, Florman S, Block A, Di Russo G, Xing J, Garg P, Grinyó J. A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). *Am J Transplant* 2010; **10**: 547-557 [PMID: 20415898 DOI: 10.1111/j.1600-6143.2010.03016.x]

22 **Florman S**, Becker T, Bresnahan B, Chevaile-Ramos A, DeCarvalho D, Muehlbacher F, O'Connell P, Duan T, Agarwal M, Larsen C. Three year outcomes by donor type in phase III studies of belatacept vs cyclosporine in kidney transplantation (BENEFIT & BENEFIT-EXT). *Am J Transplant* 2011; **11** Suppl S2: abstract 229

23 **Ferguson R**, Grinyó J, Vincenti F, Kaufman DB, Woodle ES, Marder BA, Citterio F, Marks WH, Agarwal M, Wu D, Dong Y, Garg P. Immunosuppression with belatacept-based, corticosteroid-avoiding regimens in de novo kidney transplant recipients. *Am J Transplant* 2011; **11**: 66-76 [PMID: 21114656

DOI: 10.1111/j.1600-6143.2010.03338.x]
24 **Rostaing L**, Massari P, Garcia VD, Mancilla-Urrea E, Nainan G, del Carmen Rial M, Steinberg S, Vincenti F, Shi R, Di Russo G, Thomas D, Grinyó J. Switching from calcineurin inhibitor-based regimens to a belatacept-based regimen in renal transplant recipients: a randomized phase II study. *Clin J Am Soc Nephrol* 2011; **6**: 430-439 [PMID: 21051752 DOI: 10.2215/CJN.05840710]
25 **Vanrenterghem Y**, Bresnahan B, Campistol J, Durrbach A, Grinyó J, Neumayer HH, Lang P, Larsen CP, Mancilla-Urrea E, Pestana JM, Block A, Duan T, Glicklich A, Gujrathi S, Vincenti F. Belatacept-based regimens are associated with improved cardiovascular and metabolic risk factors compared with cyclosporine in kidney transplant recipients (BENEFIT and BENEFIT-EXT studies). *Transplantation* 2011; **91**: 976-983 [PMID: 21372756 DOI: 10.1097/TP.0b013e31820c10eb]
26 **Rostaing L**, Neumayer HH, Reyes-Acevedo R, Bresnahan B, Florman S, Vitko S, Heifets M, Xing J, Thomas D, Vincenti F. Belatacept-versus cyclosporine-based immunosuppression in renal transplant recipients with pre-existing diabetes. *Clin J Am Soc Nephrol* 2011; **6**: 2696-2704 [PMID: 21921152 DOI: 10.2215/CJN.00270111]
27 **Oura T**, Yamashita K, Suzuki T, Fukumori D, Watanabe M, Hirokata G, Wakayama K, Taniguchi M, Shimamura T, Miura T, Okimura K, Maeta K, Haga H, Kubota K, Shimizu A, Sakai F, Furukawa H, Todo S. Long-term hepatic allograft acceptance based on CD40 blockade by ASKP1240 in non-human primates. *Am J Transplant* 2012; **12**: 1740-1754 [DOI: 10.1111/j.1600-6143.2012.04014.x]
28 **Goldwater R**, Keirns J, Blahunka P, First R, Sawamoto T, Zhang W, Kowalski D, Kaibara A, Holman J. A phase 1, randomized ascending single-dose study of antagonist anti-human CD40 ASKP1240 in healthy subjects. *Am J Transplant* 2013; **13**: 1040-1046 [PMID: 23356210 DOI: 10.1111/ajt.12082.]
29 **Vincenti F**, Yang H, Klintmalm G, Steinberg S, Wang L, Zhang W, Conkle A, Blahunka P, First R, Holman J. Clinical Outcomes in a Phase 1b, Randomized, Double-Blind, Parallel Group, Placebo-Controlled, Single-Dose Study of ASKP1240 in De Novo Kidney Transplantation. *Am J Transpl* 2013; **13** Suppl 5: abstr 181
30 **Jordan SC**, Vo AA, Tyan D, Nast CC, Toyoda M. Current approaches to treatment of antibody-mediated rejection. *Pediatr Transplant* 2005; **9**: 408-415 [PMID: 15910400 DOI: 10.1111/j.1399-3046.2005.00363.x]
31 **Rocha PN**, Butterly DW, Greenberg A, Reddan DN, Tuttle-Newhall J, Collins BH, Kuo PC, Reinsmoen N, Fields T, Howell DN, Smith SR. Beneficial effect of plasmapheresis and intravenous immunoglobulin on renal allograft survival of patients with acute humoral rejection. *Transplantation* 2003; **75**: 1490-1495 [PMID: 12792502 DOI: 10.1097/01.TP.0000060252.57111.AC]
32 **Becker YT**, Becker BN, Pirsch JD, Sollinger HW. Rituximab as treatment for refractory kidney transplant rejection. *Am J Transplant* 2004; **4**: 996-1001 [PMID: 15147435 DOI: 10.1111/j.1600-6143.2004.00454.x]
33 **Montgomery R**, Simpkins C, Zachary A. Anti-CD20 rescue therapy for kidneys undergoing antibody-mediated rejection. *Am J Transplant* 2004; **4**: abstract 258
34 **Locke JE**, Zachary AA, Haas M, Melancon JK, Warren DS, Simpkins CE, Segev DL, Montgomery RA. The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. *Am J Transplant* 2007; **7**: 842-846 [PMID: 17391127 DOI: 10.1111/j.1600-6143.2006.01709.x]
35 **Faguer S**, Kamar N, Guilbeaud-Frugier C, Fort M, Modesto A, Mari A, Ribes D, Cointault O, Lavayssière L, Guitard J, Durand D, Rostaing L. Rituximab therapy for acute humoral rejection after kidney transplantation. *Transplantation* 2007; **83**: 1277-1280 [PMID: 17496547 DOI: 10.1097/01.tp.0000261113.30757.d1]
36 **Blume OR**, Yost SE, Kaplan B. Antibody-mediated rejection: pathogenesis, prevention, treatment, and outcomes. *J Transplant* 2012; **2012**: 201754 [PMID: 22545199 DOI: 10.1155/2012/201754]
37 **Roberts DM**, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. *Transplantation* 2012; **94**: 775-783 [PMID: 23032865]
38 **Lefaucheur C**, Nochy D, Andrade J, Verine J, Gautreau C, Charron D, Hill GS, Glotz D, Suberbielle-Boissel C. Comparison of combination Plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. *Am J Transplant* 2009; **9**: 1099-1107 [PMID: 19422335 DOI: 10.1111/j.1600-6143.2009.02591.x]
39 **Schmidt N**, Alloway RR, Walsh RC, Sadaka B, Shields AR, Girnita AL, Hanseman DJ, Woodle ES. Prospective evaluation of the toxicity profile of proteasome inhibitor-based therapy in renal transplant candidates and recipients. *Transplantation* 2012; **94**: 352-361 [PMID: 22836132 DOI: 10.1097/TP.0b013e318257acf6]
40 **Trivedi HL**, Terasaki PI, Feroz A, Everly MJ, Vanikar AV, Shankar V, Trivedi VB, Kaneku H, Idica AK, Modi PR, Khemchandani SI, Dave SD. Abrogation of anti-HLA antibodies via proteasome inhibition. *Transplantation* 2009; **87**: 1555-1561 [PMID: 19461494 DOI: 10.1097/TP.0b013e3181a4b91b]
41 **Everly MJ**, Terasaki PI, Hopfield J, Trivedi HL, Kaneku H. Protective immunity remains intact after antibody removal by means of proteasome inhibition. *Transplantation* 2010; **90**: 1493-1498 [PMID: 21042236 DOI: 10.1097/TP.0b013e3181ff87b1]
42 **Everly MJ**, Everly JJ, Susskind B, Brailey P, Arend LJ, Alloway RR, Roy-Chaudhury P, Govil A, Mogilishetty G, Rike AH, Cardi M, Wadih G, Tevar A, Woodle ES. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation* 2008; **86**: 1754-1761 [PMID: 19104417 DOI: 10.1097/TP.0b013e318190af83]
43 **Flechner SM**, Fatica R, Askar M, Stephany BR, Poggio E, Koo A, Banning S, Chiesa-Vottero A, Srinivas T. The role of proteasome inhibition with bortezomib in the treatment of antibody-mediated rejection after kidney-only or kidney-combined organ transplantation. *Transplantation* 2010; **90**: 1486-1492 [PMID: 21042239 DOI: 10.1097/TP.0b013e3181fdd9b0]
44 **Walsh RC**, Everly JJ, Brailey P, Rike AH, Arend LJ, Mogilishetty G, Govil A, Roy-Chaudhury P, Alloway RR, Woodle ES. Proteasome inhibitor-based primary therapy for antibody-mediated renal allograft rejection. *Transplantation* 2010; **89**: 277-284 [PMID: 20145517 DOI: 10.1097/TP.0b013e3181c6ff8d]
45 **Sberro-Soussan R**, Zuber J, Suberbielle-Boissel C, Candon S, Martinez F, Snanoudj R, Rabant M, Pallet N, Nochy D, Anglicheau D, Leruez M, Loupy A, Thervet E, Hermine O, Legendre C. Bortezomib as the sole post-renal transplantation desensitization agent does not decrease donor-specific anti-HLA antibodies. *Am J Transplant* 2010; **10**: 681-686 [PMID: 20121729 DOI: 10.1111/j.1600-6143.2009.02968.x]
46 **Nigos JG**, Arora S, Nath P, Hussain SM, Marcus RJ, Ko TY, Sureshkumar KK. Treatment of antibody-mediated rejection in kidney transplant recipients: a single-center experience with a bortezomib-based regimen. *Exp Clin Transplant* 2012; **10**: 609-613 [PMID: 23216567 DOI: 10.6002/ect.2012.0131]
47 **Tzvetanov I**, Spaggiari M, Joseph J, Jeon H, Thielke J, Oberholzer J, Benedetti E. The use of bortezomib as a rescue treatment for acute antibody-mediated rejection: report of three cases and review of literature. *Transplant Proc* 2012; **44**: 2971-2975 [PMID: 23195008 DOI: 10.1016/j.transproceed.2012.02.037]
48 **Gheith O**, Al-Otaibi T, Nampoory N, Halim M, Nair P, Saied

T, Al-Waheeb S, Muzeirei I, Ibraheim M. Effective therapy for acute antibody-mediated rejection with mild chronic changes: case report and review of the literature. *Exp Clin Transplant* 2012; **10**: 406-409 [PMID: 22746156 DOI: 10.6002/ect.2011.0153]

49 **Sureshkumar KK**, Hussain SM, Marcus RJ, Ko TY, Khan AS, Tom K, Vivas CA, Parris G, Jasnosz KM, Thai NL. Proteasome inhibition with bortezomib: an effective therapy for severe antibody mediated rejection after renal transplantation. *Clin Nephrol* 2012; **77**: 246-253 [PMID: 22377258]

50 **Hardinger KL**, Murillo D. The influence of bortezomib on donor specific antibody reduction in patients with antibody mediated rejection. *Clin Transpl* 2011: 401-408 [PMID: 22755438]

51 **Westphal S**, Hansson S, Stelin G, Holgersson J, Mjörnstedt L, Friman S. Successful treatment of severe ABO antibody-mediated rejection using bortezomib: a case report. *Transplant Proc* 2013; **45**: 1213-1215 [PMID: 23622662 DOI: 10.1016/j.transproceed.2012.10.013]

52 **Waiser J**, Budde K, Schütz M, Liefeldt L, Rudolph B, Schönemann C, Neumayer HH, Lachmann N. Comparison between bortezomib and rituximab in the treatment of antibody-mediated renal allograft rejection. *Nephrol Dial Transplant* 2012; **27**: 1246-1251 [PMID: 21852274 DOI: 10.1093/ndt/gfr465]

53 **Rovira J**, Cid J, Gutiérrez-García G, Pereira A, Fernández-Avilés F, Rosiñol L, Martínez C, Carreras E, Urbano A, Rovira M, Lozano M. Fatal immune hemolytic anemia following allogeneic stem cell transplantation: report of 2 cases and review of literature. *Transfus Med Rev* 2013; **27**: 166-170 [PMID: 23562007 DOI: 10.1016/j.tmrv.2013.02.004]

54 **Roumenina LT**, Loirat C, Dragon-Durey MA, Halbwachs-Mecarelli L, Sautes-Fridman C, Fremeaux-Bacchi V. Alternative complement pathway assessment in patients with atypical HUS. *J Immunol Methods* 2011; **365**: 8-26 [PMID: 21215749 DOI: 10.1016/j.jim.2010.12.020]

55 **Châtelet V**, Lobbedez T, Frémeaux-Bacchi V, Ficheux M, Ryckelynck JP, Hurault de Ligny B. Eculizumab: safety and efficacy after 17 months of treatment in a renal transplant patient with recurrent atypical hemolytic-uremic syndrome: case report. *Transplant Proc* 2010; **42**: 4353-4355 [PMID: 21168697 DOI: 10.1016/j.transproceed.2010.09.125]

56 **Al-Akash SI**, Almond PS, Savell VH, Gharaybeh SI, Hogue C. Eculizumab induces long-term remission in recurrent post-transplant HUS associated with C3 gene mutation. *Pediatr Nephrol* 2011; **26**: 613-619 [PMID: 21125405 DOI: 10.1007/s00467-010-1708-6]

57 **Kavanagh D**, Richards A, Goodship T, Jalanko H. Transplantation in atypical hemolytic uremic syndrome. *Semin Thromb Hemost* 2010; **36**: 653-659 [PMID: 20865642 DOI: 10.1055/s-0030-1262887]

58 **Zimmerhackl LB**, Hofer J, Cortina G, Mark W, Würzner R, Jungraithmayr TC, Khursigara G, Kliche KO, Radauer W. Prophylactic eculizumab after renal transplantation in atypical hemolytic-uremic syndrome. *N Engl J Med* 2010; **362**: 1746-1748 [PMID: 20445192 DOI: 10.1056/NEJMc1001060]

59 **Peffault de Latour R**, Xhaard A, Fremeaux-Bacchi V, Coppo P, Fischer AM, Helley D, Socié G. Successful use of eculizumab in a patient with post-transplant thrombotic microangiopathy. *Br J Haematol* 2013; **161**: 279-280 [PMID: 23294015 DOI: 10.1111/bjh.12202]

60 **Xie L**, Nester CM, Reed AI, Zhang Y, Smith RJ, Thomas CP. Tailored eculizumab therapy in the management of complement factor H-mediated atypical hemolytic uremic syndrome in an adult kidney transplant recipient: a case report. *Transplant Proc* 2012; **44**: 3037-3040 [PMID: 23195022 DOI: 10.1016/j.transproceed.2012.07.141]

61 **Zuber J**, Le Quintrec M, Krid S, Bertoye C, Gueutin V, Lahoche A, Heyne N, Ardissino G, Chatelet V, Noël LH, Hourmant M, Niaudet P, Frémeaux-Bacchi V, Rondeau E, Legendre C, Loirat C; French Study Group for Atypical HUS. Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. *Am J Transplant* 2012; **12**: 3337-3354

62 **Alachkar N**, Bagnasco SM, Montgomery RA. Eculizumab for the treatment of two recurrences of atypical hemolytic uremic syndrome in a kidney allograft. *Transpl Int* 2012; **25**: e93-e95 [PMID: 22591029 DOI: 10.1111/j.1432-2277.2012.01497.x]

63 **Krid S**, Roumenina LT, Beury D, Charbit M, Boyer O, Frémeaux-Bacchi V, Niaudet P. Renal transplantation under prophylactic eculizumab in atypical hemolytic uremic syndrome with CFH/CFHR1 hybrid protein. *Am J Transplant* 2012; **12**: 1938-1944 [PMID: 22494769 DOI: 10.1111/j.1600-6143.2012.04051.x]

64 **Wilson CH**, Brown AL, White SA, Goodship TH, Sheerin NS, Manas DM. Successful treatment of de novo post-transplant thrombotic microangiopathy with eculizumab. *Transplantation* 2011; **92**: e42-e43 [PMID: 21989273 DOI: 10.1097/TP.0b013e318230c0bd]

65 **Hadaya K**, Ferrari-Lacraz S, Fumeaux D, Boehlen F, Toso C, Moll S, Martin PY, Villard J. Eculizumab in acute recurrence of thrombotic microangiopathy after renal transplantation. *Am J Transplant* 2011; **11**: 2523-2527 [DOI: 10.1111/j.1600-6143.2011.03696.x]

66 **Chandran S**, Baxter-Lowe L, Olson JL, Tomlanovich SJ, Webber A. Eculizumab for the treatment of de novo thrombotic microangiopathy post simultaneous pancreas-kidney transplantation--a case report. *Transplant Proc* 2011; **43**: 2097-2101 [PMID: 21693335 DOI: 10.1016/j.transproceed.2011.02.064]

67 **Lonze BE**, Dagher NN, Simpkins CE, Locke JE, Singer AL, Segev DL, Zachary AA, Montgomery RA. Eculizumab, bortezomib and kidney paired donation facilitate transplantation of a highly sensitized patient without vascular access. *Am J Transplant* 2010; **10**: 2154-2160 [PMID: 20636451 DOI: 10.1111/j.1600-6143.2010.03191.x]

68 **Cohney SJ**, Hughes P, Rosemary M, Walker RG, Cantwell L, Vanhardeveld E, et al. C5 inhibition with eculizumab to prevent antibody mediated rejection (AbMR) in patients with donor specific anti-HLA antibody (DSAb) and a positive cross match. *Am J Transplant* 2011; **11** Suppl S2: abstr 1557

69 **Stegall MD**, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, Cosio FG, Gandhi MJ, Kremers W, Gloor JM. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405-2413 [DOI: 10.1111/j.1600-6143.2011.03757.x]

70 **Biglarnia AR**, Nilsson B, Nilsson T, von Zur-Mühlen B, Wagner M, Berne C, Wanders A, Magnusson A, Tufveson G. Prompt reversal of a severe complement activation by eculizumab in a patient undergoing intentional ABO-incompatible pancreas and kidney transplantation. *Transpl Int* 2011; **24**: e61-e66 [PMID: 21696455 DOI: 10.1111/j.1432-2277.2011.01290.x]

71 **Stewart ZA**, Collins TE, Schlueter AJ, Raife TI, Holanda DG, Nair R, Reed AI, Thomas CP. Case report: Eculizumab rescue of severe accelerated antibody-mediated rejection after ABO-incompatible kidney transplant. *Transplant Proc* 2012; **44**: 3033-3036 [DOI: 10.1016/j.transproceed.2012.03.053]

72 **Galliford J**, Lawrence C, Willicombe M, Chan K, Roufosse C, McLean A, Cairns T, Cook HT. Reversal of refractory acute antibody mediated rejection with eculizumab. *Am J Transplant* 2011; **11** Suppl S2: abstr 1098

73 **González-Roncero F**, Suñer M, Bernal G, Cabello V, Toro M, Pereira P, Angel Gentil M. Eculizumab treatment of acute antibody-mediated rejection in renal transplantation: case reports. *Transplant Proc* 2012; **44**: 2690-2694 [PMID: 23146495 DOI: 10.1016/j.transproceed.2012.09.038]

74 **Kocak B**, Arpali E, Demiralp E, Yelken B, Karatas C, Gorcin S, Gorgulu N, Uzunalan M, Turkmen A, Kalayoglu M.

Eculizumab for salvage treatment of refractory antibody-mediated rejection in kidney transplant patients: case reports. *Transplant Proc* 2013; **45**: 1022-1025 [DOI: 10.1016/j.transproceed.2013.02.062]

75 **Locke JE**, Magro CM, Singer AL, Segev DL, Haas M, Hillel AT, King KE, Kraus E, Lees LM, Melancon JK, Stewart ZA, Warren DS, Zachary AA, Montgomery RA.The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am J Transplant* 2009; **9**: 231-235 [DOI: 10.1111/j.1600-6143.2008.02451.x]

76 ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000-2013

77 Genentech, Inc. (2009-04-08). "Genentech Announces Voluntary Withdrawal of Raptiva from the U.S. Market". Press release. Available from: URL: http://www.drugs.com/news/genentech-announces-voluntary-raptiva-u-s-market-17125.html

78 **Vincenti F**, Mendez R, Pescovitz M, Rajagopalan PR, Wilkinson AH, Butt K, Laskow D, Slakey DP, Lorber MI, Garg JP, Garovoy M. A phase I/II randomized open-label multicenter trial of efalizumab, a humanized anti-CD11a, anti-LFA-1 in renal transplantation. *Am J Transplant* 2007; **7**: 1770-1777 [PMID: 17564637 DOI: 10.1111/j.1600-6143.2007.01845.x]

79 **Bashir SJ**, Maibach HI. Alefacept (Biogen). *Curr Opin Investig Drugs* 2001; **2**: 631-634 [PMID: 11569937]

80 **Weaver TA**, Charafeddine AH, Agarwal A, Turner AP, Russell M, Leopardi FV, Kampen RL, Stempora L, Song M, Larsen CP, Kirk AD. Alefacept promotes co-stimulation blockade based allograft survival in nonhuman primates. *Nat Med* 2009; **15**: 746-749 [PMID: 19584865 DOI: 10.1038/nm.1993]

81 Astellas. (2009-12-15). "Voluntary US Market Discontinuation of Amevive (alefacept)". Press release. Available from: URL: http://www.amevive.com/. Retrieved June 6, 2013

82 **Rostaing L**, Charpentier B, Glyda M, Rigotti P, Hettich F, Franks B, Houbiers JG, First R, Holman JM. Alefacept Combined With Tacrolimus, Mycophenolate Mofetil and Steroids in De Novo Kidney Transplantation: A Randomized Controlled Trial. *Am J Transplant* 2013; **13**: 1724-1733 [PMID: 23730730 DOI: 10.1111/ajt.12303]

83 **Budde K**, Sommerer C, Becker T, Asderakis A, Pietruck F, Grinyo JM, Rigotti P, Dantal J, Ng J, Barten MJ, Weber M. Sotrastaurin, a novel small molecule inhibiting protein kinase C: first clinical results in renal-transplant recipients. *Am J Transplant* 2010; **10**: 571-581 [PMID: 20121745 DOI: 10.1111/j.1600-6143.2009.02980.x]

84 Transplantation: Development of sotrastaurin halted in kidney transplantation. *Nat Rev Nephrol* 2013 May 28; Epub ahead of print [DOI: 10.1038/nrneph.2013.87]

85 **Friman S**, Arns W, Nashan B, Vincenti F, Banas B, Budde K, Cibrik D, Chan L, Klempnauer J, Mulgaonkar S, Nicholson M, Wahlberg J, Wissing KM, Abrams K, Witte S, Woodle ES. Sotrastaurin, a novel small molecule inhibiting protein-kinase C: randomized phase II study in renal transplant recipients. *Am J Transplant* 2011; **11**: 1444-1455 [PMID: 21564523 DOI: 10.1111/j.1600-6143.2011.03538.x]

86 **Tedesco-Silva H**, Kho MM, Hartmann A, Vitko S, Russ G, Rostaing L, Budde K, Campistol JM, Eris J, Krishnan I, Gopalakrishnan U, Klupp J. Sotrastaurin in calcineurin inhibitor-free regimen using everolimus in de novo kidney transplant recipients. *Am J Transplant* 2013; **13**: 1757-1768 [PMID: 23659755 DOI: 10.1111/ajt.12255]

87 **Russ GR**, Tedesco-Silva H, Kuypers DR, Cohney S, Langer RM, Witzke O, Eris J, Sommerer C, von Zur-Mühlen B, Woodle ES, Gill J, Ng J, Klupp J, Chodoff L, Budde K. Efficacy of sotrastaurin plus tacrolimus after de novo kidney transplantation: randomized, phase II trial results. *Am J Transplant* 2013; **13**: 1746-1756 [PMID: 23668931 DOI: 10.1111/ajt.12251]

88 **Busque S**, Leventhal J, Brennan DC, Steinberg S, Klintmalm G, Shah T, Mulgaonkar S, Bromberg JS, Vincenti F, Hariharan S, Slakey D, Peddi VR, Fisher RA, Lawendy N, Wang C, Chan G. Calcineurin-inhibitor-free immunosuppression based on the JAK inhibitor CP-690,550: a pilot study in de novo kidney allograft recipients. *Am J Transplant* 2009; **9**: 1936-1945 [PMID: 19660021 DOI: 10.1111/j.1600-6143.2009.02720.x]

89 **Vincenti F**, Tedesco Silva H, Busque S, O'Connell P, Friedewald J, Cibrik D, Budde K, Yoshida A, Cohney S, Weimar W, Kim YS, Lawendy N, Lan SP, Kudlacz E, Krishnaswami S, Chan G. Randomized phase 2b trial of tofacitinib (CP-690,550) in de novo kidney transplant patients: efficacy, renal function and safety at 1 year. *Am J Transplant* 2012; **12**: 2446-2456 [PMID: 22682022 DOI: 10.1111/j.1600-6143.2012.04127.x]

90 **Busque S**, Vincenti F, Tedesco Silva H, O'Connell P, Tortorici M, Lawendy N, Wang N, Chan G. Adverse events of over-immunosuppression are dependent on tofacitinib exposure in kidney transplant (KT) patients. *Am J Transpl* 2013; **13** Suppl 5: abstr 182

P- Reviewers: Shou FZ, Trimarchi H **S- Editor**: Song XX
L- Editor: A **E- Editor:** Yan JL

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.78

World J Transplant 2013 December 24; 3(4): 78-90
ISSN 2220-3230 (online)

REVIEW

Exercise after heart transplantation: An overview

Kari Nytrøen, Lars Gullestad

Kari Nytrøen, Lars Gullestad, Department of Cardiology, Oslo University Hospital, Rikshospitalet, 0424 Oslo, Norway
Kari Nytrøen, Lars Gullestad, Faculty of Medicine, University of Oslo, 0316 Oslo, Norway
Author contributions: Both Nytrøen K and Gullestad L contributed to this paper.
Correspondence to: Dr. Kari Nytrøen, Department of Cardiology, Oslo University Hospital, Rikshospitalet, Potbox 4950 Nydalen, 0424 Oslo, Norway. kari.nytroen@medisin.uio.no
Telephone: +47-95189935 Fax: +47-23073917
Received: June 13, 2013 Revised: July 15, 2013
Accepted: July 23, 2013
Published online: December 24, 2013

Abstract

While life expectancy is greatly improved after a heart transplant, survival is still limited, and compared to the general population, the exercise capacity and health-related quality of life of heart transplant recipients are reduced. Increased exercise capacity is associated with a better prognosis. However, although several studies have documented positive effects of exercise after heart transplantation (HTx), little is known about the type, frequency and intensity of exercise that provides the greatest health benefits. Moreover, the long-term effects of exercise on co-morbidities and survival are also unclear. Exercise restrictions apply to patients with a denervated heart, and for decades, it was believed that the transplanted heart remained denervated. This has since been largely disproved, but despite the new knowledge, the exercise restrictions have largely remained, and up-to-date guidelines on exercise prescription after HTx do not exist. High-intensity, interval based aerobic exercise has repeatedly been documented to have superior positive effects and health benefits compared to moderate exercise. This applies to both healthy subjects as well as in several patient groups, such as patients with metabolic syndrome, coronary artery disease or heart failure. However, whether the effects of this type of exercise are also applicable to heart transplant populations has not yet been fully established. The purpose of this article is to give an overview of the current knowledge about the exercise capacity and effect of exercise among heart transplant recipients and to discuss future exercise strategies.

Key words: Heart transplant; Exercise; Exercise capacity; Muscle strength; Maximum oxygen uptake; Denervation; Reinnervation; Cardiac allograft vasculopathy; Quality of life

Core tip: It is time to rethink exercise strategies among heart transplant populations. Chronotropic incompetence is not necessarily a factor that limits exercise capacity in heart transplant recipients, and the exercise restrictions that have traditionally been applied to patients with a denervated heart can be disregarded. High-intensity, interval-based aerobic exercise is superior to moderate exercise in patients with coronary artery disease and heart failure, and the positive effects of this type of exercise seem to also be largely reproducible among heart transplant recipients.

Nytrøen K, Gullestad L. Exercise after heart transplantation: An overview. *World J Transplant* 2013; 3(4): 78-90 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/78.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.78

INTRODUCTION

Heart transplantation (HTx) gives numerous patients with end-stage heart disease a second chance at life. However, although life expectancy is greatly improved, survival is reduced, mainly due to the increased frequency of late complications. Additionally, the patient's exercise capacity and health-related quality of life (HRQoL) are reduced compared with the general population[1]. Exercise capacity improves after a HTx when compared with end-stage heart failure[2-8], but continues to be subnormal when

compared with age-matched values in healthy individuals. In most studies, the peak oxygen uptake (VO_{2peak}) levels range from 50% to 70% of the general population (Table 1), and a reduced VO_{2peak} level is generally associated with a poorer prognosis[9]. Only a few studies have reported individuals reaching close to normal VO_{2peak} levels[10,11]. Both central hemodynamics and peripheral physiological abnormalities may explain the reduced exercise capacity of these patients (Table 2). These factors include reduced cardiac output due to chronotropic incompetence or reduced stroke volume, peripheral abnormalities, including reduced muscle strength and oxidative capacity, and abnormal blood supply due to impaired vasodilatory capacity or capillary density[12].

While several studies have demonstrated an effect of aerobic exercise after HTx, the majority of studies used a protocol consisting of moderate training (Table 3). Traditionally, mainly due to chronotropic incompetence because of denervation, HTx recipients have not been exposed to interval-based exercise with higher intensity because it has been considered "unphysiological". High-intensity interval training (HIT) has repeatedly proven to be a highly efficient form of exercise for improving the physical capacity of both normal subjects and patients with coronary artery disease (CAD) and heart failure[13-15]. While the exact results in various patient groups are varied, HIT has demonstrated improvements in both central and peripheral factors, such as stroke volume, left ventricle (LV) remodeling, blood volume and flow, blood pressure, endothelial function, biochemical markers, skeletal muscle function and HRQoL[14-16]. However, except for two small studies published in 2004 and 2011[11,16], HIT has not been used as an intervention among HTx recipients. A systematic review and meta-analysis regarding the effects of exercise in solid organ transplant recipients were recently published[17], concluding that "exercise training is a promising but unproven intervention for improving cardiovascular outcomes of solid organ transplant recipients". Because the existing randomized controlled trials (RCTs) are small and of relatively short duration, more large-scale RCTs are urgently needed to develop clear and evidence-based guidelines regarding exercise prescriptions after HTx.

The most recent RCT assessing effect of exercise after HTx[18], which was also the largest to date, was published too late to be included in that systematic review regarding the effects of exercise in solid organ transplant recipients[17], but the study is presented in Table 3 and will be referred to throughout this article.

TRANSPLANTED, DENERVATED HEART

In contrast to the chronotropic response of a normal heart to exercise, a newly transplanted heart is denervated, which causes higher resting heart rate (HR) and reduced HR response (chronotropic incompetence)[8]. The HR response during exercise is mainly controlled by catecholamines from the adrenal glands, resulting in a significantly slower increase of the HR at onset of exercise, a reduced peak HR, and a delayed return towards resting values after cessation of exercise[4,8,19-21]. It is a common belief that this slow HR is of great importance when designing rehabilitation programs early after HTx, especially during the very first year. An improved HR response to exercise has been demonstrated during the first year after surgery[21], but it is unclear if this is due to reinnervation, and if so, what the functional importance of possible reinnervation is[22-24].

EXERCISING WITH A TRANSPLANTED HEART

Knowledge about the denervated heart is important in order to adjust the exercise protocol to achieve the optimal effect of physical exercise. There are several small studies which have shown that aerobic exercise gives a higher exercise capacity in HTx recipients[3,5,6,25-28]. The exercise protocols used, which have mainly consisted of steady-state training with moderate intensity, have shown positive effects[3,5,6,26-28]. However, the increase in exercise capacity and the VO_{2peak} levels reached are moderate[3,5,6,26-28].

Several reports have been published on the effects of rehabilitation and exercise in non-transplant patients. The main conclusion is that high-intensity, aerobic training, especially interval-based training, is a favorable type of exercise that yields improvements in both peripheral and central factors[13,14,29]. Wisløff *et al*[14] showed that interval training improved VO_{2peak} with 46% in patients with heart failure, but it has been unclear whether this type of exercise is suitable for HTx patients.

It is assumed that the delayed HR response after HTx is a limitation in regard to adapting to interval training. Presently, it is commonly believed that because of the slow HR of these patients, the session should begin with a thorough warm-up period, which should be followed by steady-state (Steady-state training refers to no rapid changes in intensity or exercising with an even HR) aerobic exercise. Although the HR response to exercise improves with time after HTx, the prevailing opinion is that these patients should not participate in interval training. This considerably limits their possibilities in joining existing rehabilitation programs in their home environment. Additionally, it has not yet been thoroughly investigated if this form of exercise is really unsuitable for this group of patients.

MECHANISMS OF REDUCED EXERCISE CAPACITY AMONG HEART TRANSPLANT RECIPIENTS

Both central hemodynamic and peripheral physiological factors most likely contribute to the reduced exercise capacity in HTx recipients (Table 2). The central factors

Table 1 Summary of some of the observational studies describing the VO_{2peak} levels in heart transplant recipients

Study	*n*	Mean age (yr)	Mean time after HTx	VO_{2peak}: mL/kg per minute or L/min	VO_{2peak} (% of age-predicted value)	Percent of age-predicted HR_{max} or actual HR_{max} (bpm)
Renlund *et al*[123]	110	51 ± 10	26 mo	17.7 ± 0.3 mL	64% ± 1%	85%
Mandak *et al*[51]	60	52 ± 10	1 yr	16.2 ± 3.8 mL	NA	137 ± 24
Osada *et al*[124]	56	50 ± 12	3 yr	20.0 ± 5.0 mL	70% ± 17%	88% ± 11%
Notarius *et al*[12]	12	51 ± 4	8 mo	17.3 ± 1.7 mL	57%	147 ± 7
Douard *et al*[30]	85	52 ± 12	0-100 mo	21.1 ± 6 mL	NA	85% ± 13%
Squires *et al*[24]	95	48 ± 14	1 yr	19.9 ± 4.8 mL	61% ± 15%	138 ± 22
Gullestad *et al*[48]	174	51 ± 1	3.5 yr	19.4 ± 0.4 mL	70% ± 1%	146 ± 2
Myers *et al*[125]	47	47 ± 12	4.8 yr	9.4 ± 2.6 mL	59% ± 14%	129 ± 18
Schmid *et al*[126]	17	58 ± 13	65 ± 27	20.9 ± 5.2 mL	NA	136 ± 12
Richard *et al*[127]	7	40 ± 13	2 yr	NA	101% ± 12%	93% ± 9%
Carter *et al*[2]	47	48	5 yr	16.1 ± 0.5 mL	51% ± 1.5%	74% ± 1%
Ulubay *et al*[118]	7	43 ± 14	19 mo	1.45 ± 0.33 L	NA	114 ± 41

HTx: Heart transplant; HR_{max}: Maximum achieved heart rate; bpm: Beats per minute; NA: Not available.

Table 2 Possible mechanisms associated with reduced exercise capacity in heart transplant recipients

- Central cardiac factors
  - Reduced cardiac output
    - Chronotropic incompetence
    - Reduced stroke volume
      - Systolic dysfunction
      - Diastolic dysfunction
  - Pulmonary dysfunction
    - Pulmonary hypertension
    - Lung disease
    - Pulmonary congestion
- Peripheral factors
  - Decreased skeletal muscle function
    - Reduced muscle mass
    - Reduced muscle strength
    - Reduced capillary density
    - Reduced oxidative capacity
    - Reduced mitochondrial function
    - Corticosteroid induced myopathy
  - Impaired vasodilatory capacity
    - Endothelial dysfunction
  - Deconditioning
- Potential factors contributing to reduced exercise capacity
  - Increasing age
  - Donor age
  - Donor match
  - Ischemic time
  - Pre transplant de-conditioning
  - Primary diagnosis
  - Co-morbidities
  - Smoking
  - Cultural differences
  - Gender differences
  - Anxiety and depression
  - Socio-economic status
  - Reduced health-related quality of life

include chronotropic incompetence, impaired LV function or greater arteriovenous oxygen difference, while peripheral limitations include reduced muscle mass, anabolic resistance due to reduced muscle strength and oxidative capacity, or abnormal blood supply due to impaired vasodilatory capacity and capillary density. Several factors specific for HTx patients, such as immunosuppressive regimens, donor age, and ischemic time, as well as general factors, such as smoking status, prolonged deconditioning, co-morbidities, socio-economic status, and cultural differences, may contribute to their reduced performance[7,12,20,30-33] (Table 2).

HIT

The American College of Sports Medicine[34] and American Heart Association[35] recommend exercising with an intensity between 50% and 90% of maximum VO_2, which refers to approximately 60%-95% of the maximum HR. In comparison to the detailed prescription of different medications, this is a very imprecise recommendation. As such, it causes difficulties for both the health personnel who are obligated to give advice based on these recommendations, and the patients who are trying to carry out these vague exercise prescriptions. One of the reasons for the imprecise recommendations is that there has been uncertainty and disagreement regarding how VO_{2peak} improves most efficiently. The majority of researchers in the field now agree that the major factor limiting an individual's VO_{2peak} is the stroke volume. Given that maximum HR cannot be increased, the stroke volume is the limiting (and only) factor that, through exercise, may improve cardiac output[36]. It is reasonable to think that exercising at a near to maximum stroke volume would give the best results. The previous belief was that maximum stroke volume was reached at approximately 50%-70% of maximum HR[37,38], and this is still stated in most textbooks[36], even though it was shown as early as in the 1960s that stroke volume does not necessarily plateau in this range[39]. Additional and more recent research has documented, both in untrained, moderate and well trained subjects, that the stroke volume often does not reach a plateau until the HR is close to its maximum[40-44]. This has not been documented in patients with CAD, but several studies of high-intensity exercise interventions have documented a superior effect of such exercise compared to exercise at a moderate intensity, both in patients with heart failure and CAD[13,14,45-47].

Table 3 Summary of randomized controlled trials investigating the effect of exercise in heart transplant recipients

Study	*n*, mean age (yr)	Mean time after HTx	Intervention	Mean change in VO_{2peak} (mL/kg per minute) within groups	Mean change in VO_{2peak} (mL/kg per minute) between groups
Kobashigawa *et al*[3]	27, 52	1 mo	6 mo partly supervised rehabilitation program *vs* controls CG Walking: cycling and upper and lower limb exercises for 30 min × 1-3/wk	Ex: 9.2 → 13.6 Con: 10.4 → 12.3	2.5
Tegtbur *et al*[26]	32, 55	5 yr	1 yr home-based exercise program *vs* controls Cycling every other day for 1 yr at 80%-90% of maximum HR	Ex: 18.6 → 20.0 Con: 18.9 → 19.0	1.3
Bernardi *et al*[6]	24, 52	6 mo	6 mo home training *vs* controls Cycling at 60%-70% of VO_{2peak} 30 min × 5/wk × 6 mo	Ex: 14.9 → 19.6 Con: 14.3 → 15.6	3.4
Karapolat *et al*[5]	28, 42	1.5 yr	8 wk supervised hospital training *vs* home-based training 1.5 h of multiple exercises: including aerobic exercise for 30 min at 60%-70% of VO_{2peak} × 3/wk The controls received written guidelines on exercises and a walking program	Ex: 16.7 → 19.5 Con: 20.1 → 19.5	3.4
Wu *et al*[27]	37, 56	2 yr	8 wk home training *vs* controls Strength training and aerobic exercise at 60%-70% of VO_{2peak}: 35-40 min × 3/wk	Ex: 12.1 → 13.2 Con: 13.7 → 13.2	1.6
Haykowsky *et al*[25]	43, 59	5 yr	12 wk aerobic/strength training *vs* controls First 8 wk: continuous aerobic exercise at 60%-80% of VO_{2peak}: 30-45 min × 2/wk Continuous aerobic training at 80% of VO_{2peak}, 45 min × 2/wk and bicycle interval training for 30 s at 90%-100% of VO_{2peak}: followed by 60 s rest for 10-25 reps × 2/wk in the final 4 wk Resistance training at 50% of 1RM: 10-15 reps × 1-2 sets × 4 exercises × 2/wk for 12 wk	Ex: 21.2 → 24.7 Con: 18.2 → 18.2	3.5
Hermann *et al*[16]	27, 50	7 yr	8 wk high-intensity interval training *vs* controls Interval blocks of 4 min/2 min/30 s: corresponding to 80%: 85% and 90% of VO_{2peak}: respectively: and recovery periods of ½ min: and finally staircase running at 80% of VO_{2peak}: followed by recovery walking 60 min × 3/wk	Ex: 23.9 → 28.3 Con: 24.6 → 23.4	5.6
Nytrøen *et al*[18]	48, 51	4 yr	1 yr high-intensity interval training *vs* controls 4 interval blocks of 4 min each performed at 91% of peak HR: with 3 min active recovery periods between each block 3 periods of 8 wk distributed throughout 1 yr with exercise 3/wk for a total of 72 exercise sessions	Ex: 27.7 → 30.9 Con: 28.5 → 28.0	3.6

HTx: Heart transplant; NT: No training; Ex: Exercise group; Con: Control group; 1RM: 1 repetition maximum; HR: Heart rate.

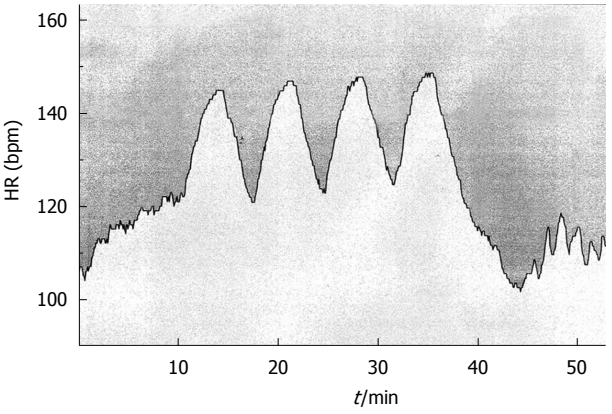

Figure 1 Example of a 4 x 4 high-intensity interval training session performed by a heart transplant recipient.

Most individuals should be able to reach an intensity of approximately 90%-95% of their maximum HR within 1-2 min. Based on this, the leading research community of this field in Norway (Norwegian University of Science and Technology, Trondheim) have proposed and documented that 4 exercise bouts of 4 min each (4 × 4) (Figure 1), with an active break in between, is an exercise prescription that is highly effective and works very well[38].

HIT IN HEART TRANSPLANT RECIPIENTS

As shown in Table 3, only a limited number of RCTs have investigated the effect of exercise in HTx recipients. Furthermore, most of these studies compared some form of rehabilitation/exercise program with a control group that was not receiving any specific type of exercise strategy, and most of the exercises performed in the intervention groups were performed in-hospital and at a moderate intensity. It is challenging to conduct large-scale, high-quality, in-hospital exercise studies because the HTx recipients often live very far away from the transplantation centers, and it is difficult and expensive to recruit enough patients to participate in a long-term exercise program very far away from their home. Thus, a training program that is close to the patients' home might be more desirable. We tested such an approach in a study investigating the effect of HIT in HTx recipients[18], and even though Norway is a small country with a very small

HTx population, this was manageable because we designed the intervention to be carried out individually, in each of the patients' home communities.

Each participant was assigned to a local physical therapist who cooperated closely with our hospital and the people in charge of the trial. The exercise intervention, which lasted for 1 year, was divided into three main, intensive exercise periods lasting 8 wk each. After each 8-wk period, the project group and the local physical therapist discussed the results and the improvements in each individual patient thus far and made plans for the next intensive exercise period. The evaluation of the training period included HR analyses from the HR monitors and exercise logs, re-testing of the patient's maximum HR and adjustment of the desired training zones, if necessary.

When the study first started, we were anxious to see whether this fairly ambitious exercise intervention would and could be sustained by the participants for a full year. Of the 52 patients who were initially recruited, 48 completed the follow-up, and the results exceeded our expectations, showing that a mean of 96% of the planned exercise sessions were completed at the target intensity throughout the year, without any adverse events. Four patients did not complete the study due to other reasons. Thus, we feel that it is safe to conclude that this form of exercise is both highly applicable and safe in stable, long-term HTx recipients[18]. Our results are in accordance with the only other two existing studies that introduced HIT to HTx recipients[11,16], but future large-scale studies are needed to confirm the observed effects on peak exercise capacity and determine the mechanisms by which this occurs. Additionally, the studies should examine whether HIT has beneficial effects besides improving exercise capacity, such as in reducing transplant related complications. Furthermore, HIT also needs to be compared to other exercise strategies and not only control groups receiving no particular training regimen.

EFFECT OF EXERCISE IN HEART TRANSPLANT RECIPIENTS

The first RCT investigating the effect of exercise in HTx recipients was published by Kobashigawa *et al*[3]. The trial included patients who were 1 mo post HTx and compared a 6-mo rehabilitation program to a control group receiving no specific exercise strategy. The mean change between the groups at follow-up was 3.4 mL/kg per minute ($P < 0.001$), but the VO_{2peak} level reached at the end of the intervention was low compared to the values predicted for healthy subjects of similar age and sex. This was also reflected in most of the subsequent trials. Table 3 describes the details of the published RCTs during the period from 1999 to 2012. The mean VO_{2peak} values reached after the exercise interventions ranged from 13.2 to 28.3 mL/kg per minute in the various studies, and the mean change between the control and exercise groups ranges from 1.3 to 5.6 mL/kg per minute. Except from the two previously mentioned randomized HIT-studies[16,18], the mean intensity of the aerobic training was reported to be 60%-80% of VO_{2peak} in the majority of the studies, the exercise duration ranged from 30 min to 1.5 h, 2-5 times per week for 8 wk up to 1 year, the mean time after HTx ranged from 1 mo up to 7 years, and the number of participants ranged from 24 to 43 (Table 3).

Based on the great differences between these trials, the somewhat inconsistent results and the lack of a control group undergoing a different exercise strategy in most of the RCTs, it can only be concluded that exercise improves VO_{2peak}; it is not yet possible to state which type of exercise, intensity and duration gives optimal results, although a HIT-intervention seems to be favorable.

Most of the studies describing VO_{2peak} levels in HTx recipients reported that the levels to be between 50% and 70% of predicted values[2,7,20,48]. The highest level reported was from a study carried out at our center[18]. The exercise group improved their VO_{2peak} level from 27.7 to 30.9 mL/kg per minute, corresponding to 80% to 89% of predicted values in healthy subjects (Table 1). The high baseline VO_{2peak} values may have been a result of selection bias, based on the design of the study, the exercise intervention and the inclusion criteria. However, considering the wide range of VO_{2peak} values (from 13.9 to 44.0 mL/kg per minute, corresponding to 46% to 130% of predicted) and the normally distributed data, this suggests a heterogeneous group rather than a selected group of well fit HTx recipients, and the data might mirror the stable and healthy Norwegian HTx population quite well. Our high levels are supported by another recent Nordic RCT investigating the effect of high-intensity exercise in HTx recipients[16]. In that study, the authors demonstrated higher than average VO_{2peak} values, with a baseline value of 23.9 improving to 28.3 mL/kg per minute at follow-up, even with a considerably higher mean time after HTx of 7 years *vs* 4 years in our study. It is unclear whether this could be a reflection that Scandinavian HTx recipients have levels above average, or if it is due to type of test protocol used in other studies and/or uncertainty about whether maximal intensity really was reached during the exercise test. However, we believe that HIT interventions[16,18] likely induced a greater effect than moderate training. This is in accordance with previous studies among patients with CAD[13] and left ventricular dysfunction[14], which have used comparable HIT protocols.

At follow-up, the mean change in VO_{2peak} between the groups in our study[18] was 3.6 mL/kg per minute. This is similar to three of the other RCTs involving moderate training that are presented in Table 3[6,25,28]. However, it is important to note that two of these studies[6,28] had considerably lower baseline VO_{2peak} values and that it is well known that subjects with low initial VO_{2peak} levels easily gain greater improvements than those with fairly high baseline values[49]. Haykowsky *et al*[25] demonstrated a similar improved mean VO_{2peak} follow-up value in the exercise group (24.7 mL/kg per minute *vs* 30.9 mL/kg per minute) and reported a similar mean change between the groups of 3.5 mL/kg per minute. This exercise inter-

vention[25] also included elements of HIT, which makes a more suitable comparison for our study. Haykowsky's study[25], which was published in April 2009, the same year we started including patients in our RCT, was then the RCT with the highest demonstrated VO_{2peak} improvement among HTx recipients. In 2011, during the course of our study, Hermann *et al*[16] published the results from their study, demonstrating an overwhelming difference of 5.6 mL/kg per minute between the exercise group and the control group after 8 wk of exercising 3 times per week. Although it is questionable why and how the control group reduced their VO_{2peak} level from 24.6 to 23.4 mL/kg per minute in only 8 wk, which contributed to the large mean difference between the groups, the exercise group still had a remarkable mean improvement of 4.4 mL/kg per minute, substantially supporting HIT as a highly effective form of exercise in long-term HTx recipients. Similarly, our study supports HIT as a safe, applicable and effective form of exercise, and the field is now ready and in need of future studies investigating the effects of HIT compared to other exercise interventions. The timing is also important, as the health benefits may be even greater if the intervention is started earlier, that is, shortly after HTx.

Chronotropic responses and reinnervation

In the 1990s, it was widely believed that "total denervation persists in the human heart following cardiac transplantation"[50] and "the lack of alteration in the HR response over time, suggests that no significant functional reinnervation occurs"[51]. This was the common belief in most research communities in early studies among HTx recipients. However, in last decade, a body of evidence has repudiated this statement. Nevertheless, several studies evaluating sympathetic and parasympathetic reinnervation have yielded somewhat contradicting results, especially with respect to possible parasympathetic reinnervation[52-56]. The evidence of sympathetic reinnervation seems to be more frequent and certain, but is inconsistent in nature[57-63]. Multiple different direct and indirect methods of evaluating reinnervation, such as HR variability analysis[64], cardial release of noradrenalin[22], positron-emission tomography[65] and the evaluation of the chronotropic response as a sign of functional reinnervation, have also yielded varying results[66].

The normalization of the chronotropic responses is associated with functional reinnervation and better exercise capacity[6,30,57-60,65]. Along the same lines, the reduced exercise capacity in HTx recipients is generally associated with chronotropic incompetence due to denervation. Multiple studies showing partial normalization of the HR response have reported discrepant results regarding the degree of normalization and percent of subjects developing normalization, in addition to great differences according the time after HTx when the improved chronotropic response is confirmed. Bernardi *et al*[6] showed that autonomic nervous control can be improved by physical training, while others have proposed that reinnervation occurs independently over time[4,61,67]. Richard *et al*[10] and Pokan *et al*[11] have shown peak HR values close to or above age-predicted in HTx recipients. These findings are supported by a study from our center[21], in which we documented a high degree of normalization of chronotropic responses within 6 mo after HTx. This occurred earlier and with a higher degree of normalization than demonstrated by others[2,24,62,67-69]. The high degree of normalization during the first year after HTx[21] was confirmed in a different long-term HTx population in the previously discussed HIT-intervention study[18]. The exercise group in this study significantly improved their peak HR from 154 to 163 bpm, whereas the control group remained unchanged (154 bpm *vs* 153 bpm). This finding supports Bernardi *et al*[6], but it is still unclear whether time alone may result in the normalization of chronotropic responses or whether it occurs in combination with exercise or others factors.

Because it has been assumed that chronotropic incompetence in HTx recipients is a limitation towards adapting to interval training, and because the patients have atypical central and peripheral responses to exercise, previously described training regimens have mostly consisted of moderate, steady-state intensity exercise[4,17,32] (Table 3). Only a few previous studies have described close to normal HR responses in HTx recipients[10,11]. These, together with our recent studies[18,21], have provided increasing evidence suggesting that HR response is not a limiting factor for exercise capacity in the majority of HTx patients. Hopefully, this finding will contribute to minimizing the persistent exercise restrictions that apply to patients with denervated hearts. A future challenge is to identify which factors influence the reinnervation process and why it is inconsistent and does not occur in all HTx subjects.

The absence of parasympathetic activity is clearly evident in the denervated heart, which has an elevated resting HR, often more than 100 bpm[70]. Resting HR reflects vagal tone and HR recovery is known to be a marker of parasympathetic activity[71-74] while HR increase at onset of exercise and peak HR reflect sympathetic activity[19,70,75]. The improved HR increase and close to normal peak HR and HR reserve observed in several studies[10,11,18,21] support the notion of functional, sympathetic reinnervation. In one of our studies[21], we found improved HR recovery, a marker of parasympathetic activity[70,72,73,76], thus suggesting parasympathetic reinnervation. In contrast, persistent elevated resting HR[21] was not consistent with vagal reinnervation. Although we also found a significant lower resting HR in another study[18], suggesting improved vagal reinnervation, our results only confirm the inconsistencies in the literature regarding reinnervation in general.

Central vs peripheral effects of exercise

Pulmonary diffusion, cardiac output and blood volume are regarded as the main central limitations to oxygen delivery, while the role of peripheral factors limiting VO_{2peak} has been an object of greater discussion[77]. While it is agreed upon that VO_{2peak} is dependent on the interac-

tion between O_2-transport and muscle (mitochondrial) consumption of O_2, there is disagreement as to which of these is the main determinant[77]. The results vary largely in trained *vs* sedentary subjects or in different patient groups *vs* normal subjects. In athletes, as in patients with chronic lung disease, pulmonary diffusion seems to be the greatest limitation. In healthy, untrained subjects and in patients with heart failure, the principal limiting factor is cardiac output, often combined with skeletal muscle limitations[78]. In HTx recipients, it is assumed that reduced exercise capacity is due to a combination of central and peripheral physiological abnormalities[12,79], but the mechanisms behind this subnormal capacity is not completely understood. Thus, we initially hypothesized in our recent trial[18,80] that a possible increase in VO_{2peak} after a HIT intervention would be positively affected by both central and peripheral factors. To study myocardial performance, we performed thorough examinations of the systolic and diastolic function of all participants using newer echocardiographic techniques, both at rest and during sub-maximal exercise (bicycle ergometer). In contrast to the documented improved cardiac function of HIT in patients with cardiovascular diseases in general, we found, rather surprisingly, no alterations of clinical importance, either in cardiac systolic or diastolic function as assessed by echocardiographic measurements[80]. This suggests that HTx recipients respond differently to HIT than other groups of patients. Muscle diffusion capacity, mitochondrial enzyme levels and capillary density are other potential peripheral sites for VO_{2peak} limitation[33,77]. Although most research supports cardiovascular delivery as the central component in VO_{2peak}, the importance of skeletal muscle function should not be underestimated[33]. Borrelli *et al*[82] found that the main gain in VO_{2peak} was at the peripheral level. In accordance with this, we found that muscular exercise capacity and amount of body fat were strong factors predicting VO_{2peak} in a group of HTx recipients[83]. These findings were confirmed in a follow-up study[18], where the same peripheral factors made the most significant contributions to the improvement in VO_{2peak}. These findings suggests that peripheral muscular and metabolic alterations have a substantial impact on the aerobic exercise capacity in HTx recipients and that they may have a greater impact than cardiac limitations[33].

However, despite the absence of detectable echocardiographic improvements in the current study[80], the HIT group demonstrated a higher O_2-pulse and lower HR at sub-maximal exercise levels, which indicate increased stroke volume. In addition, the chronotropic response index (CRI), which reflects both the maximum HR and the resting HR, significantly increased from 0.89 to 0.95. These somewhat contradictory findings regarding cardiac function may be explained by the small number of observations, possibly causing a type 2 statistical error. Echocardiographic measurements during peak exercise and a higher number of observations could reveal an undetected improvement in cardiac function in future studies.

Because of the initially high and close to normal CRI, and a mean time of 4 years after HTx, we did not expect any notable improvement in the chronotropic responses. Nevertheless, at follow-up, the HIT group showed a significant increase in their CRI as a result of both a lower resting HR and a higher peak HR[18]. However, it is still unclear whether improved autonomic nervous control is a result of exercise[6] or if it occurs independently, as a result of time[4,63]. As previously mentioned, a large number of studies have documented partial sympathetic reinnervation. This appears to be greatest during the first few years after a HTx and then gradually decreases[4,59]. The findings in our study[18] indicate that the peak HR, despite a close to normal level at baseline (4 years post HTx), can still be influenced by intensive exercise and fully reach, or even exceed, the expected maximum HR. A total of 30% of the patients in the current study had an expected maximum HR > 100% (range 100%-111%).

In summary, it seems quite clear that HTx recipients respond differently to exercise than other patient groups and that the beneficial training effects in a number of studies[18,25,82] predominantly rely on peripheral mechanisms, especially muscular exercise capacity. However, some of the findings in our study[18] also suggest some improvements in cardiac function and that autonomic regulation is improved and close to normal. Further investigation is needed to establish why and how the transplanted hearts respond differently to high-intensity exercise than normal subjects and other patients groups.

EFFECT OF EXERCISE ON TRANSPLANT RELATED COMPLICATIONS

It is well known that many HTx recipients develop a number of complications, such as cardiac allograft vasculopathy (CAV), graft failure, renal failure, cancer, gout, metabolic disturbances, including hyperlipidemia and diabetes mellitus, and reduced HRQoL. These may all contribute to increased morbidity and reduced survival. In general, exercise might have beneficial effects on several of these parameters in the general population and in different patients groups. However, little is known about the role of exercise on transplant related complications. The few RCTs that have been published[17,32] (Table 3) have mainly documented the effects of exercise on exercise capacity, muscle strength, body composition, endothelial function, some inflammatory biomarkers and HRQoL. However, detailed knowledge regarding other effects of exercise, such as on metabolic disturbances, renal function and co-morbidities, does not exist. Studies describing factors associated with exercise capacity also provide limited and uncertain information because the specific factors considered varies greatly[30,31,48,83].

We recently examined the effect of HIT on CAV[84]. CAV (Figure 2) is a rapidly progressive form of atherosclerosis that occurs in HTx recipients and involves diffuse thickening and occlusion of the coronary arteries[85]. The classic early sign of CAV, intimal thickening, is present in approximately 58% of the arteries during the first year

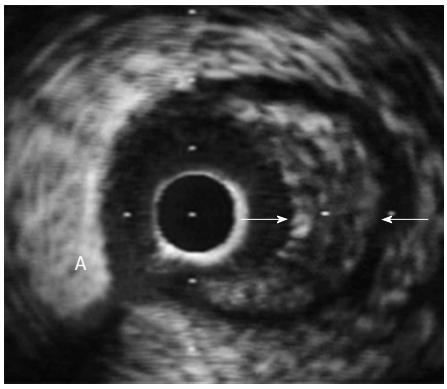

Figure 2 Intravascular ultra sound increases the sensitivity for early diagnosis of cardiac allograft vasculopathy compared to traditional angiography. Diffuse intimal thickening (area between the arrows) can be accurately identified by intra vascular ultra sound in contrast to angiography where only the lumen size is visible.

after HTx[86,87]. Later, luminal stenosis of the epicardial branches and occlusion of the smaller arteries develop, resulting secondarily in myocardial ischemia and infarction[88]. Treating CAV is a great therapeutic challenge, and it is the leading cause of late graft-loss and death among HTx patients. The only real cure for severe CAV is retransplantation[85,88]. Because of denervation, myocardial ischemia may be asymptomatic in many cases, and CAV can only be diagnosed by coronary angiography or intravascular ultra sound (IVUS). IVUS increases the sensitivity for early diagnosis compared to traditional angiography[85] (Figure 2).

Existing therapy options, both for the prevention and treatment of CAV, including immunosuppressive drugs and statin therapy early after HTx, have so far not given the desired results[88-90]. In clinical practice, prophylaxis of CAV involves modification of general risk factors, such as smoking, obesity, diabetes and hypertension, as well as the implementation of physical activity[91]. However, although the atheroprotective effect of exercise and the effect of physical activity on established CAD is well documented[92-94], with HIT being reported to have a more pronounced effect[14,38], knowledge about the specific effect of exercise on CAV is very limited.

CAV continues to limit the long term success of HTx; CAV is, in addition to malignancy, the most important causes of death in patients who survive the first year after HTx. In our recent study[84], we demonstrated, using serial IVUS measurements, that the progression of CAV was reduced by more than 50% in the HIT group compared to the control group[84]. Although no previous reports on the effect of exercise on CAV progression in HTx patients exist, our finding is in accordance with the effect of exercise on progression of coronary atherosclerosis among patients with CAD[95,96], specifically, an increased threshold for chest pain[97]. In a recent study by Yoshikawa *et al*[95], it was shown that a high VO_{2peak} level was associated with healthier tissue composition and less coronary plaque in patients with CAD. Additionally, a recent review article summarizing the impact of exercise training on arterial wall thickness concluded that exercise can decrease arterial wall thickness in subjects with CAD or with cardiovascular risk factors, as well as in healthy subjects[96]. However, what type of exercise, frequency, duration and intensity that gives the best results remain to be determined.

In contrast to most studies investigating the effect of exercise on cardiovascular health, our study[84] had a long-term, high-intensity exercise intervention. If the effect on CAV is due to this mode of exercise is uncertain and needs to be confirmed in future studies. However, we believe that a high-intensity program is needed, given that the control group in our study must be considered a moderate training group as they performed a considerable amount of exercise during the study period. Specifically, only 33% exercised little or not at all, and 67% exercised 2 times or more per week.

Several mechanisms are involved in the initiation and progression of CAV, including innate and adaptive immune responses, as well as risk factors, such as smoking, hypertension, hyperglycemia, hypercholesterolemia, body mass index (BMI) and metabolic disturbances[98]. In our study, we found that the progression of CAV, as assessed by an increase in percent atheroma volume (PAV) > 1.5%, was associated with a significantly higher mean change in weight, BMI and visceral fat. Because a high BMI and visceral fat are associated with increased inflammation, which is a well-known factor contributing to the development of endothelial dysfunction, atherosclerosis and CAV[99], a possible mechanism by which HIT affects CAV progression could be mediated by a reduction in the inflammatory burden. This is consistent with studies among patients with CAD that have suggested that the effect of exercise on atherosclerosis may be explained, to some extent, by its influence on metabolism and its anti-inflammatory effects[100,101]. However, in the present study[84], other than significantly lower IL-8 level at follow-up (within-group), a numerically lower level of C-reactive protein (CRP) and a numerically higher level of IL-6 in the HIT group, there was no clear effect of HIT on the differential expression inflammatory mediators between the groups. This contrasts the findings of Hermann *et al*[16], who carried out a comparable HIT intervention and found a significant reduction of CRP in the HTx exercise group. The reason for the discrepant finding is unclear but could be related to patient population, duration of exercise or timing of blood sampling. We cannot rule out that exercise could have a beneficial effect on vascular inflammation that is not associated with a systemic inflammatory response. Lower increases in PAV were associated with a reduction in visceral fat, as well as BMI and weight[84], which could be due to an effect of HIT on adipocyte-derived mediators. This needs to be clarified in future studies.

Health related quality of life

Studies investigating HRQoL after HTx have clearly demonstrated that HTx recipients have significantly improved HRQoL compared to the pre transplant stage[102-107]. These studies have mainly used generic questionnaires or

a combination of generic and disease specific questionnaires[102-107]. Several studies have reported that the improved HRQoL also remains high in the long-term after HTx[102,103,108-111]. In contrast, we previously found reduced HRQoL among HTx patients in the long term after surgery compared with newly transplanted patients[112]. Compared with HRQoL scores in general populations, HTx populations demonstrate varied results. Some studies report no HRQoL differences between HTx recipients and the general population[106,110,111], whereas others have reported that HTx populations have significantly lower HRQoL scores compared with a reference population[103,108,109,112-116]. Furthermore, reduced HRQoL is associated with anxiety and depression after HTx[105,107,114,117].

In contrast to the previous study from our center demonstrating a higher frequency of depression and anxiety[112], both groups in the most recent study from our center had high scores on HRQoL and no symptoms of anxiety or depression at baseline[18]. This might be due to our inclusion criteria, allowing only stable and healthy HTx recipients to participate. The high baseline HRQoL scores among both groups limited the possibility of revealing an actual effect of exercise in this area, but despite the ceiling-effect, there was a clear trend towards a better overall HRQoL in the HIT group compared to the control group in all domains of SF36[18]. This was also confirmed by a significantly higher rating in the HIT group on the VAS scale, which assessed their subjective opinion on whether participation in the HIT intervention generated positive influences on their general health[18]. This supports previously documented evidence on the association between increased exercise capacity and better HRQoL[105,118-122].

SUMMARY AND IMPLICATIONS FOR FOLLOW-UP AND FUTURE RESEARCH

In the past few years, there has been increasing focus on using physical exercise as a tool in both the primary and secondary prophylaxis of cardiovascular diseases, which is the main cause of sickness and death in the western world. Despite the increased focus and great benefits of regular exercise, it is still underutilized as a therapeutic intervention.

Traditionally, several exercise restrictions have applied to the transplanted, denervated heart, which seems to be based more on caution than scientific evidence. The time seems to be right for rethinking the use of exercise among HTx recipients and to offer an "up to date" physical training principle to this group of patients who are presently not recommended to participate in HIT programs.

The high degree of normalization of chronotropic responses among HTx recipients should be a major factor in support of reducing the exercise restrictions that have applied to the denervated heart. Accumulating evidence suggest that chronotropic incompetence is not a factor limiting exercise capacity in the majority of HTx recipients and that HIT is a feasible, safe and effective way to improve exercise capacity and general health in stable, long term HTx recipients. This type of exercise should be introduced and used more frequently among a broader audience. However, the transplanted heart seems to respond differently to this type of exercise, resulting mainly in peripheral improvements rather than improved cardiac function. Larger studies and more basic research are needed to investigate these mechanisms. Future research is also needed to determine if the positive effects on CAV are reproducible, to examine which mechanisms cause these effects and to determine whether such an intervention has an effect on long term survival. The important question regarding optimal timing for introducing HIT after HTx also needs to be assessed. At present there is not (yet) sufficient evidence to conclude that HIT is superior to moderate exercise in HTx-recipients.

REFERENCES

1 **Stehlik J**, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dobbels F, Kirk R, Rahmel AO, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: Twenty-eighth Adult Heart Transplant Report--2011. *J Heart Lung Transplant* 2011; **30**: 1078-1094 [PMID: 21962016 DOI: 10.1016/j.healun.2011.08.003]

2 **Carter R**, Al-Rawas OA, Stevenson A, Mcdonagh T, Stevenson RD. Exercise responses following heart transplantation: 5 year follow-up. *Scott Med J* 2006; **51**: 6-14 [PMID: 16910044 DOI: 10.1258/rsmsmj.51.3.6]

3 **Kobashigawa JA**, Leaf DA, Lee N, Gleeson MP, Liu H, Hamilton MA, Moriguchi JD, Kawata N, Einhorn K, Herlihy E, Laks H. A controlled trial of exercise rehabilitation after heart transplantation. *N Engl J Med* 1999; **340**: 272-277 [PMID: 9920951 DOI: 10.1056/NEJM199901283400404]

4 **Kavanagh T**. Exercise rehabilitation in cardiac transplantation patients: a comprehensive review. *Eura Medicophys* 2005; **41**: 67-74 [PMID: 16175772]

5 **Karapolat H**, Eyigor S, Zoghi M, Yagdi T, Nalbantgil S, Durmaz B, Ozbaran M. Effects of cardiac rehabilitation program on exercise capacity and chronotropic variables in patients with orthotopic heart transplant. *Clin Res Cardiol* 2008; **97**: 449-456 [PMID: 18317667 DOI: 10.1007/s00392-008-0648-7]

6 **Bernardi L**, Radaelli A, Passino C, Falcone C, Auguadro C, Martinelli L, Rinaldi M, Viganò M, Finardi G. Effects of physical training on cardiovascular control after heart transplantation. *Int J Cardiol* 2007; **118**: 356-362 [PMID: 17050012 DOI: 10.1016/j.ijcard.2006.07.032]

7 **Squires RW**. Exercise therapy for cardiac transplant recipients. *Prog Cardiovasc Dis* 2011; **53**: 429-436 [PMID: 21545929 DOI: 10.1016/j.pcad.2011.03.010]

8 **Braith RW**, Edwards DG. Exercise following heart transplantation. *Sports Med* 2000; **30**: 171-192 [PMID: 10999422 DOI: 10.2165/00007256-200030030-00003]

9 **Kavanagh T**, Mertens DJ, Hamm LF, Beyene J, Kennedy J, Corey P, Shephard RJ. Prediction of long-term prognosis in 12169 men referred for cardiac rehabilitation. *Circulation* 2002; **106**: 666-671 [PMID: 12163425 DOI: 10.1161/01.CIR.0000024413.15949.ED]

10 **Richard R**, Verdier JC, Duvallet A, Rosier SP, Leger P, Nignan A, Rieu M. Chronotropic competence in endurance trained heart transplant recipients: heart rate is not a limiting factor for exercise capacity. *J Am Coll Cardiol* 1999; **33**: 192-197 [PMID: 9935029 DOI: 10.1016/S0735-1097(98)00513-0]

11 **Pokan R**, Von Duvillard SP, Ludwig J, Rohrer A, Hofmann P, Wonisch M, Smekal G, Schmid P, Pacher R, Bachl N. Effect of high-volume and -intensity endurance training in heart transplant recipients. *Med Sci Sports Exerc* 2004; **36**: 2011-2016 [PMID: 15570133 DOI: 10.1249/01.MSS.0000147630.71210.06]

12 **Notarius CF**, Levy RD, Tully A, Fitchett D, Magder S. Cardi-

ac versus noncardiac limits to exercise after heart transplantation. *Am Heart J* 1998; **135**: 339-348 [PMID: 9489986 DOI: 10.1016/S0002-8703(98)70103-6]
13 **Rognmo Ø**, Hetland E, Helgerud J, Hoff J, Slørdahl SA. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur J Cardiovasc Prev Rehabil* 2004; **11**: 216-222 [PMID: 15179103 DOI: 10.1097/01.hjr.0000131677.96762.0c]
14 **Wisløff U**, Støylen A, Loennechen JP, Bruvold M, Rognmo Ø, Haram PM, Tjønna AE, Helgerud J, Slørdahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen Ø, Skjaerpe T. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* 2007; **115**: 3086-3094 [PMID: 17548726 DOI: 10.1161/CIRCULATIONAHA.106.675041]
15 **Arena R**, Myers J, Forman DE, Lavie CJ, Guazzi M. Should high-intensity-aerobic interval training become the clinical standard in heart failure? *Heart Fail Rev* 2013; **18**: 95-105 [PMID: 22791516 DOI: 10.1007/s10741-012-9333-z]
16 **Hermann TS**, Dall CH, Christensen SB, Goetze JP, Prescott E, Gustafsson F. Effect of high intensity exercise on peak oxygen uptake and endothelial function in long-term heart transplant recipients. *Am J Transplant* 2011; **11**: 536-541 [PMID: 21219582 DOI: 10.1111/j.1600-6143.2010.03403.x]
17 **Didsbury M**, McGee RG, Tong A, Craig JC, Chapman JR, Chadban S, Wong G. Exercise training in solid organ transplant recipients: a systematic review and meta-analysis. *Transplantation* 2013; **95**: 679-687 [PMID: 23364480 DOI: 10.1097/TP.0b013e31827a3d3e]
18 **Nytrøen K**, Rustad LA, Aukrust P, Ueland T, Hallén J, Holm I, Rolid K, Lekva T, Fiane AE, Amlie JP, Aakhus S, Gullestad L. High-intensity interval training improves peak oxygen uptake and muscular exercise capacity in heart transplant recipients. *Am J Transplant* 2012; **12**: 3134-3142 [PMID: 22900793 DOI: 10.1111/j.1600-6143.2012.04221.x]
19 **Orizio C**, Perini R, Comandè A, Castellano M, Beschi M, Veicsteinas A. Plasma catecholamines and heart rate at the beginning of muscular exercise in man. *Eur J Appl Physiol Occup Physiol* 1988; **57**: 644-651 [PMID: 3396585 DOI: 10.1007/BF00418477]
20 **Marconi C**, Marzorati M. Exercise after heart transplantation. *Eur J Appl Physiol* 2003; **90**: 250-259 [PMID: 13680240 DOI: 10.1007/s00421-003-0952-x]
21 **Nytrøen K**, Myers J, Chan KN, Geiran OR, Gullestad L. Chronotropic responses to exercise in heart transplant recipients: 1-yr follow-up. *Am J Phys Med Rehabil* 2011; **90**: 579-588 [PMID: 21765276 DOI: 10.1097/PHM.0b013e31821f711d]
22 **Wilson RF**, Christensen BV, Olivari MT, Simon A, White CW, Laxson DD. Evidence for structural sympathetic reinnervation after orthotopic cardiac transplantation in humans. *Circulation* 1991; **83**: 1210-1220 [PMID: 2013143 DOI: 10.1161/01.CIR.83.4.1210]
23 **Kaye DM**, Esler M, Kingwell B, McPherson G, Esmore D, Jennings G. Functional and neurochemical evidence for partial cardiac sympathetic reinnervation after cardiac transplantation in humans. *Circulation* 1993; **88**: 1110-1118 [PMID: 8353872 DOI: 10.1161/01.CIR.88.3.1110]
24 **Squires RW**, Leung TC, Cyr NS, Allison TG, Johnson BD, Ballman KV, Wagner JA, Olson LJ, Frantz RP, Edwards BS, Kushwaha SS, Dearani JA, Daly RC, McGregor CG, Rodeheffer RJ. Partial normalization of the heart rate response to exercise after cardiac transplantation: frequency and relationship to exercise capacity. *Mayo Clin Proc* 2002; **77**: 1295-1300 [PMID: 12479515 DOI: 10.4065/77.12.1295]
25 **Haykowsky M**, Taylor D, Kim D, Tymchak W. Exercise training improves aerobic capacity and skeletal muscle function in heart transplant recipients. *Am J Transplant* 2009; **9**: 734-739 [PMID: 19344465 DOI: 10.1111/j.1600-6143.2008.02531.x]
26 **Tegtbur U**, Busse MW, Jung K, Pethig K, Haverich A. Time course of physical reconditioning during exercise rehabilitation late after heart transplantation. *J Heart Lung Transplant* 2005; **24**: 270-274 [PMID: 15737752 DOI: 10.1016/j.healun.2003.12.010]
27 **Wu YT**, Chien CL, Chou NK, Wang SS, Lai JS, Wu YW. Efficacy of a home-based exercise program for orthotopic heart transplant recipients. *Cardiology* 2008; **111**: 87-93 [PMID: 18376119 DOI: 10.1159/000119695]
28 **Karapolat H**, Eyigör S, Zoghi M, Yagdi T, Nalbangil S, Durmaz B. Comparison of hospital-supervised exercise versus home-based exercise in patients after orthotopic heart transplantation: effects on functional capacity, quality of life, and psychological symptoms. *Transplant Proc* 2007; **39**: 1586-1588 [PMID: 17580194 DOI: 10.1016/j.transproceed.2007.01.079]
29 **Tjønna AE**, Lee SJ, Rognmo Ø, Stølen TO, Bye A, Haram PM, Loennechen JP, Al-Share QY, Skogvoll E, Slørdahl SA, Kemi OJ, Najjar SM, Wisløff U. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation* 2008; **118**: 346-354 [PMID: 18606913 DOI: 10.1161/CIRCULATIONAHA.108.772822]
30 **Douard H**, Parrens E, Billes MA, Labbe L, Baudet E, Broustet JP. Predictive factors of maximal aerobic capacity after cardiac transplantation. *Eur Heart J* 1997; **18**: 1823-1828 [PMID: 9402458 DOI: 10.1093/oxfordjournals.eurheartj.a015178]
31 **Leung TC**, Ballman KV, Allison TG, Wagner JA, Olson LJ, Frantz RP, Edwards BS, Dearani JA, Daly RC, McGregor CG, Rodeheffer RJ. Clinical predictors of exercise capacity 1 year after cardiac transplantation. *J Heart Lung Transplant* 2003; **22**: 16-27 [PMID: 12531409 DOI: 10.1016/S1053-2498(02)00475-8]
32 **Hsieh PL**, Wu YT, Chao WJ. Effects of exercise training in heart transplant recipients: a meta-analysis. *Cardiology* 2011; **120**: 27-35 [PMID: 22094922 DOI: 10.1159/000332998]
33 **Williams TJ**, McKenna MJ. Exercise limitation following transplantation. *Compr Physiol* 2012; **2**: 1937-1979 [PMID: 23723030]
34 **American College of Sports Medicine**. ACSM's guidelines for exercise testing and prescription. 8th ed. Baltimore: Wolters Kluwer, Lippincott Williams and Wilkins, 2009: 60-104
35 **Fletcher GF**, Balady GJ, Amsterdam EA, Chaitman B, Eckel R, Fleg J, Froelicher VF, Leon AS, Piña IL, Rodney R, Simons-Morton DA, Williams MA, Bazzarre T. Exercise standards for testing and training: a statement for healthcare professionals from the American Heart Association. *Circulation* 2001; **104**: 1694-1740 [PMID: 11581152 DOI: 10.1161/hc3901.095960]
36 **Åstrand PO**, Rodahl K, Dahl HA, Strømme SB. Textbook of work physiology: physiological bases of exercise. 4th ed. Champaign: Human Kinetics Publishers, 2003
37 **Vella CA**, Robergs RA. A review of the stroke volume response to upright exercise in healthy subjects. *Br J Sports Med* 2005; **39**: 190-195 [PMID: 15793084 DOI: 10.1136/bjsm.2004.013037]
38 **Wisløff U**, Ellingsen Ø, Kemi OJ. High-intensity interval training to maximize cardiac benefits of exercise training? *Exerc Sport Sci Rev* 2009; **37**: 139-146 [PMID: 19550205 DOI: 10.1097/JES.0b013e3181aa65fc]
39 **Chapman CB**, Fisher JN, Sproule BJ. Behavior of stroke volume at rest and during exercise in human beings. *J Clin Invest* 1960; **39**: 1208-1213 [PMID: 13809220 DOI: 10.1172/JCI104136]
40 **Zhou B**, Conlee RK, Jensen R, Fellingham GW, George JD, Fisher AG. Stroke volume does not plateau during graded exercise in elite male distance runners. *Med Sci Sports Exerc* 2001; **33**: 1849-1854 [PMID: 11689734 DOI: 10.1097/00005768-200111000-00008]
41 **Gledhill N**, Cox D, Jamnik R. Endurance athletes' stroke volume does not plateau: major advantage is diastolic function. *Med Sci Sports Exerc* 1994; **26**: 1116-1121 [PMID: 7808245 DOI: 10.1249/00005768-199409000-00008]
42 **Wang E**, Solli GS, Nyberg SK, Hoff J, Helgerud J. Stroke

volume does not plateau in female endurance athletes. *Int J Sports Med* 2012; **33**: 734-739 [PMID: 22581683 DOI: 10.1055/s-0031-1301315]
43 **Krip B**, Gledhill N, Jamnik V, Warburton D. Effect of alterations in blood volume on cardiac function during maximal exercise. *Med Sci Sports Exerc* 1997; **29**: 1469-1476 [PMID: 9372484 DOI: 10.1097/00005768-199711000-00013]
44 **Martino M**, Gledhill N, Jamnik V. High VO2max with no history of training is primarily due to high blood volume. *Med Sci Sports Exerc* 2002; **34**: 966-971 [PMID: 12048323 DOI: 10.1097/00005768-200206000-00010]
45 **Kemi OJ**, Wisloff U. High-intensity aerobic exercise training improves the heart in health and disease. *J Cardiopulm Rehabil Prev* 2010; **30**: 2-11 [PMID: 20040880]
46 **Karlsen T**, Hoff J, Støylen A, Skovholdt MC, Gulbrandsen Aarhus K, Helgerud J. Aerobic interval training improves VO2 peak in coronary artery disease patients; no additional effect from hyperoxia. *Scand Cardiovasc J* 2008; **42**: 303-309 [PMID: 18609057 DOI: 10.1080/14017430802032723]
47 **Helgerud J**, Høydal K, Wang E, Karlsen T, Berg P, Bjerkaas M, Simonsen T, Helgesen C, Hjorth N, Bach R, Hoff J. Aerobic high-intensity intervals improve VO2max more than moderate training. *Med Sci Sports Exerc* 2007; **39**: 665-671 [PMID: 17414804 DOI: 10.1249/mss.0b013e3180304570]
48 **Gullestad L**, Myers J, Edvardsen T, Kjekshus J, Geiran O, Simonsen S. Predictors of exercise capacity and the impact of angiographic coronary artery disease in heart transplant recipients. *Am Heart J* 2004; **147**: 49-54 [PMID: 14691418 DOI: 10.1016/j.ahj.2003.07.013]
49 **Anderssen SA**, Strømme SB. [Physical activity and health--recommendations]. *Tidsskr Nor Laegeforen* 2001; **121**: 2037-2041 [PMID: 11875902]
50 **Shephard RJ**. Responses of the cardiac transplant patient to exercise and training. *Exerc Sport Sci Rev* 1992; **20**: 297-320 [PMID: 1623890]
51 **Mandak JS**, Aaronson KD, Mancini DM. Serial assessment of exercise capacity after heart transplantation. *J Heart Lung Transplant* 1995; **14**: 468-478 [PMID: 7654732]
52 **Uberfuhr P**, Frey AW, Fuchs A, Paniara C, Roskamm H, Schwaiger M, Reichart B. Signs of vagal reinnervation 4 years after heart transplantation in spectra of heart rate variability. *Eur J Cardiothorac Surg* 1997; **12**: 907-912 [PMID: 9489879 DOI: 10.1016/S1010-7940(97)00271-6]
53 **Beckers F**, Ramaekers D, Speijer G, Ector H, Vanhaecke J, Verheyden B, Van Cleemput J, Droogné W, Van de Werf F, Aubert AE. Different evolutions in heart rate variability after heart transplantation: 10-year follow-up. *Transplantation* 2004; **78**: 1523-1531 [PMID: 15599318 DOI: 10.1097/01.TP.0000141093.04132.41]
54 **Halpert I**, Goldberg AD, Levine AB, Levine TB, Kornberg R, Kelly C, Lesch M. Reinnervation of the transplanted human heart as evidenced from heart rate variability studies. *Am J Cardiol* 1996; **77**: 180-183 [PMID: 8546088 DOI: 10.1016/S0002-9149(96)90592-5]
55 **Arrowood JA**, Minisi AJ, Goudreau E, Davis AB, King AL. Absence of parasympathetic control of heart rate after human orthotopic cardiac transplantation. *Circulation* 1997; **96**: 3492-3498 [PMID: 9396446 DOI: 10.1161/01.CIR.96.10.3492]
56 **Uberfuhr P**, Frey AW, Reichart B. Vagal reinnervation in the long term after orthotopic heart transplantation. *J Heart Lung Transplant* 2000; **19**: 946-950 [PMID: 11044688 DOI: 10.1016/S1053-2498(00)00181-9]
57 **Lovric SS**, Avbelj V, Trobec R, Zorman D, Rakovec P, Hojker S, Gersak B, Milcinski M. Sympathetic reinnervation after heart transplantation, assessed by iodine-123 metaiodobenzylguanidine imaging, and heart rate variability. *Eur J Cardiothorac Surg* 2004; **26**: 736-741 [PMID: 15450565 DOI: 10.1016/j.ejcts.2004.07.025]
58 **Wilson RF**, Johnson TH, Haidet GC, Kubo SH, Mianuelli M. Sympathetic reinnervation of the sinus node and exercise hemodynamics after cardiac transplantation. *Circulation* 2000; **101**: 2727-2733 [PMID: 10851211 DOI: 10.1161/01.CIR.101.23.2727]
59 **Uberfuhr P**, Ziegler S, Schwaiblmair M, Reichart B, Schwaiger M. Incomplete sympathic reinnervation of the orthotopically transplanted human heart: observation up to 13 years after heart transplantation. *Eur J Cardiothorac Surg* 2000; **17**: 161-168 [PMID: 10731652 DOI: 10.1016/S1010-7940(99)00367-X]
60 **Schwaiblmair M**, von Scheidt W, Uberfuhr P, Ziegler S, Schwaiger M, Reichart B, Vogelmeier C. Functional significance of cardiac reinnervation in heart transplant recipients. *J Heart Lung Transplant* 1999; **18**: 838-845 [PMID: 10528745 DOI: 10.1016/S1053-2498(99)00048-0]
61 **Bengel FM**, Ueberfuhr P, Ziegler SI, Nekolla S, Reichart B, Schwaiger M. Serial assessment of sympathetic reinnervation after orthotopic heart transplantation. A longitudinal study using PET and C-11 hydroxyephedrine. *Circulation* 1999; **99**: 1866-1871 [PMID: 10199884]
62 **Ferretti G**, Marconi C, Achilli G, Caspani E, Fiocchi R, Mamprin F, Gamba A, Ferrazzi P, Cerretelli P. The heart rate response to exercise and circulating catecholamines in heart transplant recipients. *Pflugers Arch* 2002; **443**: 370-376 [PMID: 11810205 DOI: 10.1007/s004240100701]
63 **Bengel FM**, Ueberfuhr P, Karja J, Schreiber K, Nekolla SG, Reichart B, Schwaiger M. Sympathetic reinnervation, exercise performance and effects of beta-adrenergic blockade in cardiac transplant recipients. *Eur Heart J* 2004; **25**: 1726-1733 [PMID: 15451151 DOI: 10.1016/j.ehj.2004.07.009]
64 **Sanatani S**, Chiu C, Nykanen D, Coles J, West L, Hamilton R. Evolution of heart rate control after transplantation: conduction versus autonomic innervation. *Pediatr Cardiol* 2004; **25**: 113-118 [PMID: 14647999 DOI: 10.1007/s00246-003-0387-5]
65 **Bengel FM**, Ueberfuhr P, Schiepel N, Nekolla SG, Reichart B, Schwaiger M. Effect of sympathetic reinnervation on cardiac performance after heart transplantation. *N Engl J Med* 2001; **345**: 731-738 [PMID: 11547742 DOI: 10.1056/NEJMoa010519]
66 **Gallego-Page JC**, Segovia J, Alonso-Pulpón L, Alonso-Rodríguez M, Salas C, Ortíz-Berrocal J. Re-innervation after heart transplantation: a multidisciplinary study. *J Heart Lung Transplant* 2004; **23**: 674-682 [PMID: 15366426 DOI: 10.1016/j.healun.2003.07.011]
67 **Mercier J**, Ville N, Wintrebert P, Caillaud C, Varray A, Albat B, Thévenet A, Préfaut C. Influence of post-surgery time after cardiac transplantation on exercise responses. *Med Sci Sports Exerc* 1996; **28**: 171-175 [PMID: 8775150 DOI: 10.1097/00005768-199602000-00003]
68 **Gullestad L**, Haywood G, Ross H, Bjornerheim R, Geiran O, Kjekshus J, Simonsen S, Fowler M. Exercise capacity of heart transplant recipients: the importance of chronotropic incompetence. *J Heart Lung Transplant* 1996; **15**: 1075-1083 [PMID: 8956116]
69 **Carvalho VO**, Pascoalino LN, Bocchi EA, Ferreira SA, Guimarães GV. Heart rate dynamics in heart transplantation patients during a treadmill cardiopulmonary exercise test: a pilot study. *Cardiol J* 2009; **16**: 254-258 [PMID: 19437401]
70 **Freeman JV**, Dewey FE, Hadley DM, Myers J, Froelicher VF. Autonomic nervous system interaction with the cardiovascular system during exercise. *Prog Cardiovasc Dis* 2006; **48**: 342-362 [PMID: 16627049 DOI: 10.1016/j.pcad.2005.11.003]
71 **Cole CR**, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. *N Engl J Med* 1999; **341**: 1351-1357 [PMID: 10536127 DOI: 10.1056/NEJM199910283411804]
72 **Dogru MT**, Gunaydin S, Simsek V, Tulmac M., Guneri M. Correlation of Chronotropic Index and Heart Rate Recovery to Heart Rate Variability and Diastolic Function Values in Men. *Inter J Cardiol* 2007; 4. Available from: URL: http://is-pub.com/IJC/4/2/11549
73 **Lauer M**, Froelicher ES, Williams M, Kligfield P. Exercise

testing in asymptomatic adults: a statement for professionals from the American Heart Association Council on Clinical Cardiology, Subcommittee on Exercise, Cardiac Rehabilitation, and Prevention. *Circulation* 2005; **112**: 771-776 [PMID: 15998671 DOI: 10.1161/CIRCULATIONAHA.105.166543]

74 **Desai MY**, De la Peña-Almaguer E, Mannting F. Abnormal heart rate recovery after exercise as a reflection of an abnormal chronotropic response. *Am J Cardiol* 2001; **87**: 1164-1169 [PMID: 11356391 DOI: 10.1016/S0002-9149(01)01487-4]

75 **Londeree BR**, Moeschberger ML. Influence of age and other factors on maximal heart rate. *J Card Rehabil* 1984; **4**: 44-49

76 **Myers J**, Tan SY, Abella J, Aleti V, Froelicher VF. Comparison of the chronotropic response to exercise and heart rate recovery in predicting cardiovascular mortality. *Eur J Cardiovasc Prev Rehabil* 2007; **14**: 215-221 [PMID: 17446799 DOI: 10.1097/HJR.0b013e328088cb92]

77 **Bassett DR**, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 2000; **32**: 70-84 [PMID: 10647532 DOI: 10.1097/00005768-200001000-00012]

78 **Middlekauff HR**. Making the case for skeletal myopathy as the major limitation of exercise capacity in heart failure. *Circ Heart Fail* 2010; **3**: 537-546 [PMID: 20647489]

79 **Mettauer B**, Levy F, Richard R, Roth O, Zoll J, Lampert E, Lonsdorfer J, Geny B. Exercising with a denervated heart after cardiac transplantation. *Ann Transplant* 2005; **10**: 35-42 [PMID: 17037087]

80 **Rustad LA**, Nytrøen K, Amundsen BH, Gullestad L, Aakhus S. One year of high-intensity interval training improves exercise capacity, but not left ventricular function in stable heart transplant recipients: A randomisedcontrolled trial. *Eur J Prev Cardiol* 2012; Epub ahead of print [PMID: 23185084 DOI: 10.1177/2047487312469477]

81 **Molmen-Hansen HE**, Stolen T, Tjonna AE, Aamot IL, Ekeberg IS, Tyldum GA, Wisloff U, Ingul CB, Stoylen A. Aerobic interval training reduces blood pressure and improves myocardial function in hypertensive patients. *Eur J Prev Cardiol* 2012; **19**: 151-160 [PMID: 21450580 DOI: 10.1177/1741826711400512]

82 **Borrelli E**, Pogliaghi S, Molinello A, Diciolla F, Maccherini M, Grassi B. Serial assessment of peak VO2 and VO2 kinetics early after heart transplantation. *Med Sci Sports Exerc* 2003; **35**: 1798-1804 [PMID: 14600540 DOI: 10.1249/01.MSS.0000093610.71730.02]

83 **Nytrøen K**, Rustad LA, Gude E, Hallén J, Fiane AE, Rolid K, Holm I, Aakhus S, Gullestad L. Muscular exercise capacity and body fat predict VO2peak in heart transplant recipients. *Eur J Prev Cardiol* 2012; Epub ahead of print [PMID: 22659939 DOI: 10.1177/2047487312450540]

84 **Nytrøen K**, Annette Rustad L, Erikstad I, Aukrust P, Ueland T, Lekva T, Gude E, Wilhelmsen N, Hervold A, Aakhus S, Gullestad L, Arora S. Effect of high-intensity interval training on progression of cardiac allograft vasculopathy. *J Heart Lung Transplant* 2013 [PMID: 23906899]

85 **Avery RK**. Cardiac-allograft vasculopathy. *N Engl J Med* 2003; **349**: 829-830 [PMID: 12944567 DOI: 10.1056/NEJMp038124]

86 **Billingham ME**. The pathologic changes in long-term heart and lung transplant survivors. *J Heart Lung Transplant* 1992; **11**: S252-S257 [PMID: 1387551]

87 **Stoica SC**, Goddard M, Large SR. The endothelium in clinical cardiac transplantation. *Ann Thorac Surg* 2002; **73**: 1002-1008 [PMID: 11899159 DOI: 10.1016/S0003-4975(01)03000-4]

88 **Weis M**. Cardiac allograft vasculopathy: prevention and treatment options. *Transplant Proc* 2002; **34**: 1847-1849 [PMID: 12176600 DOI: 10.1016/S0041-1345(02)03101-9]

89 **Segovia J**, Gómez-Bueno M, Alonso-Pulpón L. Treatment of allograft vasculopathy in heart transplantation. *Expert Opin Pharmacother* 2006; **7**: 2369-2383 [PMID: 17109612 DOI: 10.1517/14656566.7.17.2369]

90 **Wang SS**. Treatment and prophylaxis of cardiac allograft vasculopathy. *Transplant Proc* 2008; **40**: 2609-2610 [PMID: 18929815 DOI: 10.1016/j.transproceed.2008.08.073]

91 **Chou NK**, Jan CF, Chi NH, Lee CM, Wu IH, Huang SC, Chen YS, Yu HY, Tsao CI, Ko WJ, Chu SH, Wang SS. Cardiac allograft vasculopathy compared by intravascular ultrasound sonography: everolimus to mycophenolate mofetil--one single-center experience. *Transplant Proc* 2012; **44**: 897-899 [PMID: 22564578 DOI: 10.1016/j.transproceed.2012.03.041]

92 **Szostak J**, Laurant P. The forgotten face of regular physical exercise: a 'natural' anti-atherogenic activity. *Clin Sci* (Lond) 2011; **121**: 91-106 [PMID: 21729002 DOI: 10.1042/CS20100520]

93 **Ahmed HM**, Blaha MJ, Nasir K, Rivera JJ, Blumenthal RS. Effects of physical activity on cardiovascular disease. *Am J Cardiol* 2012; **109**: 288-295 [PMID: 22011559 DOI: 10.1016/j.amjcard.2011.08.042]

94 **Swift DL**, Lavie CJ, Johannsen NM, Arena R, Earnest CP, O'Keefe JH, Milani RV, Blair SN, Church TS. Physical activity, cardiorespiratory fitness, and exercise training in primary and secondary coronary prevention. *Circ J* 2013; **77**: 281-292 [PMID: 23328449 DOI: 10.1253/circj.CJ-13-0007]

95 **Yoshikawa D**, Ishii H, Kurebayashi N, Sato B, Hayakawa S, Ando H, Hayashi M, Isobe S, Okumura T, Hirashiki A, Takeshita K, Amano T, Uetani T, Yamada S, Murohara T. Association of cardiorespiratory fitness with characteristics of coronary plaque: assessment using integrated backscatter intravascular ultrasound and optical coherence tomography. *Int J Cardiol* 2013; **162**: 123-128 [PMID: 21636151 DOI: 10.1016/j.ijcard.2011.05.047]

96 **Thijssen DH**, Cable NT, Green DJ. Impact of exercise training on arterial wall thickness in humans. *Clin Sci* (Lond) 2012; **122**: 311-322 [PMID: 22150253 DOI: 10.1042/CS20110469]

97 **Wienbergen H**, Hambrecht R. Physical exercise and its effects on coronary artery disease. *Curr Opin Pharmacol* 2013; **13**: 218-225 [PMID: 23333176 DOI: 10.1016/j.coph.2012.12.003]

98 **Schmauss D**, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation* 2008; **117**: 2131-2141 [PMID: 18427143 DOI: 10.1161/CIRCULATIONAHA.107.711911]

99 **Weis M**, von Scheidt W. Cardiac allograft vasculopathy: a review. *Circulation* 1997; **96**: 2069-2077 [PMID: 9323100 DOI: 10.1161/01.CIR.96.6.2069]

100 **Pedersen BK**. The diseasome of physical inactivity--and the role of myokines in muscle--fat cross talk. *J Physiol* 2009; **587**: 5559-5568 [PMID: 19752112 DOI: 10.1113/jphysiol.2009.179515]

101 **Lavie CJ**, Church TS, Milani RV, Earnest CP. Impact of physical activity, cardiorespiratory fitness, and exercise training on markers of inflammation. *J Cardiopulm Rehabil Prev* 2011; **31**: 137-145 [PMID: 21427600]

102 **Burra P**, De Bona M. Quality of life following organ transplantation. *Transpl Int* 2007; **20**: 397-409 [PMID: 17403143 DOI: 10.1111/j.1432-2277.2006.00440.x]

103 **Grady KL**. Quality of life after heart transplantation: are things really better? *Curr Opin Cardiol* 2003; **18**: 129-135 [PMID: 12652219 DOI: 10.1097/00001573-200303000-00011]

104 **Martín-Rodríguez A**, Pérez-San-Gregorio MA, Díaz-Domínguez R, Pérez-Bernal J. Health-related quality of life evolution in patients after heart transplantation. *Transplant Proc* 2008; **40**: 3037-3038 [PMID: 19010184 DOI: 10.1016/j.transproceed.2008.08.118]

105 **Karapolat H**, Eyigor S, Durmaz B, Yagdi T, Nalbantgil S, Karakula S. The relationship between depressive symptoms and anxiety and quality of life and functional capacity in heart transplant patients. *Clin Res Cardiol* 2007; **96**: 593-599 [PMID: 17593317 DOI: 10.1007/s00392-007-0536-6]

106 **Kugler C**, Tegtbur U, Gottlieb J, Bara C, Malehsa D, Dierich M, Simon A, Haverich A. Health-related quality of life in long-term survivors after heart and lung transplantation: a prospective cohort study. *Transplantation* 2010; **90**: 451-457 [PMID: 20562734 DOI: 10.1097/TP.0b013e3181e72863]

107 **Evangelista LS**, Dracup K, Moser DK, Westlake C, Erickson V, Hamilton MA, Fonarow GC. Two-year follow-up of quality of

life in patients referred for heart transplant. *Heart Lung* 2005; **34**: 187-193 [PMID: 16015223 DOI: 10.1016/j.hrtlng.2004.10.004]
108 **Saeed I**, Rogers C, Murday A. Health-related quality of life after cardiac transplantation: results of a UK National Survey with Norm-based Comparisons. *J Heart Lung Transplant* 2008; **27**: 675-681 [PMID: 18503969 DOI: 10.1016/j.healun.2008.03.013]
109 **Dew MA**, Switzer GE, Goycoolea JM, Allen AS, DiMartini A, Kormos RL, Griffith BP. Does transplantation produce quality of life benefits? A quantitative analysis of the literature. *Transplantation* 1997; **64**: 1261-1273 [PMID: 9371666 DOI: 10.1097/00007890-199711150-00006]
110 **Jokinen JJ**, Hämmäinen P, Lemström KB, Lommi J, Sipponen J, Harjula AL. Association between gastrointestinal symptoms and health-related quality of life after heart transplantation. *J Heart Lung Transplant* 2010; **29**: 1388-1394 [PMID: 20851638 DOI: 10.1016/j.healun.2010.07.002]
111 **Petroski RA**, Grady KL, Rodgers S, Backer CL, Kulikowska A, Canter C, Pahl E. Quality of life in adult survivors greater than 10 years after pediatric heart transplantation. *J Heart Lung Transplant* 2009; **28**: 661-666 [PMID: 19560692 DOI: 10.1016/j.healun.2009.04.004]
112 **Sivertsen B**, Relbo A, Gullestad L, Hellesvik M, Grov I, Andreassen A, Simonsen S, Geiran O, Havik OE. [Self-assessed health and psychological symptoms after heart transplantation]. *Tidsskr Nor Laegeforen* 2007; **127**: 3198-3201 [PMID: 18084359]
113 **Politi P**, Piccinelli M, Fusar-Poli P, Klersy C, Campana C, Goggi C, Viganò M, Barale F. Ten years of "extended" life: quality of life among heart transplantation survivors. *Transplantation* 2004; **78**: 257-263 [PMID: 15280687 DOI: 10.1097/01.TP.0000133537.87951.F2]
114 **Fusar-Poli P**, Martinelli V, Klersy C, Campana C, Callegari A, Barale F, Viganò M, Politi P. Depression and quality of life in patients living 10 to 18 years beyond heart transplantation. *J Heart Lung Transplant* 2005; **24**: 2269-2278 [PMID: 16364881 DOI: 10.1016/j.healun.2005.06.022]
115 **Kugler C**, Malehsa D, Tegtbur U, Guetzlaff E, Meyer AL, Bara C, Haverich A, Strueber M. Health-related quality of life and exercise tolerance in recipients of heart transplants and left ventricular assist devices: a prospective, comparative study. *J Heart Lung Transplant* 2011; **30**: 204-210 [PMID: 20980169 DOI: 10.1016/j.healun.2010.08.030]
116 **Loge JH**, Kaasa S. Short form 36 (SF-36) health survey: normative data from the general Norwegian population. *Scand J Soc Med* 1998; **26**: 250-258 [PMID: 9868748]
117 **Grady KL**, Naftel DC, Kobashigawa J, Chait J, Young JB, Pelegrin D, Czerr J, Heroux A, Higgins R, Rybarczyk B, McLeod M, White-Williams C, Kirklin JK. Patterns and predictors of quality of life at 5 to 10 years after heart transplantation. *J Heart Lung Transplant* 2007; **26**: 535-543 [PMID: 17449426 DOI: 10.1016/j.healun.2007.01.042]
118 **Ulubay G**, Ulasli SS, Sezgin A, Haberal M. Assessing exercise performance after heart transplantation. *Clin Transplant* 2007; **21**: 398-404 [PMID: 17488391 DOI: 10.1111/j.1399-0012.2007.00658.x]
119 **Karapolat H**, Eyigor S, Durmaz B, Nalbantgil S, Yagdi T, Zoghi M. The effect of functional performance, respiratory function and osteopenia on the quality of life after heart transplantation. *Int J Cardiol* 2008; **124**: 381-383 [PMID: 17395310 DOI: 10.1016/j.ijcard.2006.12.053]
120 **Christensen SB**, Dall CH, Prescott E, Pedersen SS, Gustafsson F. A high-intensity exercise program improves exercise capacity, self-perceived health, anxiety and depression in heart transplant recipients: a randomized, controlled trial. *J Heart Lung Transplant* 2012; **31**: 106-107 [PMID: 22153554 DOI: 10.1016/j.healun.2011.10.014]
121 **Hsu CJ**, Chen SY, Su S, Yang MC, Lan C, Chou NK, Hsu RB, Lai JS, Wang SS. The effect of early cardiac rehabilitation on health-related quality of life among heart transplant recipients and patients with coronary artery bypass graft surgery. *Transplant Proc* 2011; **43**: 2714-2717 [PMID: 21911151 DOI: 10.1016/j.transproceed.2011.04.025]
122 **Buendía F**, Almenar L, Martínez-Dolz L, Sánchez-Lázaro I, Navarro J, Agüero J, Muñoz B, Sánchez-Gómez JM, Cebrian M, Salvador A. Relationship between functional capacity and quality of life in heart transplant patients. *Transplant Proc* 2011; **43**: 2251-2252 [PMID: 21839248 DOI: 10.1016/j.transproceed.2011.05.003]
123 **Renlund DG**, Taylor DO, Ensley RD, O'Connell JB, Gilbert EM, Bristow MR, Ma H, Yanowitz FG. Exercise capacity after heart transplantation: influence of donor and recipient characteristics. *J Heart Lung Transplant* 1996; **15**: 16-24 [PMID: 8820079]
124 **Osada N**, Chaitman BR, Donohue TJ, Wolford TL, Stelken AM, Miller LW. Long-term cardiopulmonary exercise performance after heart transplantation. *Am J Cardiol* 1997; **79**: 451-456 [PMID: 9052349 DOI: 10.1016/S0002-9149(96)00785-0]
125 **Myers J**, Gullestad L, Bellin D, Ross H, Vagelos R, Fowler M. Physical activity patterns and exercise performance in cardiac transplant recipients. *J Cardiopulm Rehabil* 2003; **23**: 100-106 [PMID: 12668931 DOI: 10.1097/00008483-200303000-00006]
126 **Schmid JP**, Gaillet R, Noveanu M, Mohacsi P, Saner H, Hullin R. Influence of the exercise protocol on peak VO2 in patients after heart transplantation. *J Heart Lung Transplant* 2005; **24**: 1751-1756 [PMID: 16297776 DOI: 10.1016/j.healun.2005.02.010]
127 **Richard R**, Verdier JC, Doutreleau S, Piquard F, Gény B, Rieu M. Exercise limitation in trained heart and kidney transplant recipients: central and peripheral limitations. *J Heart Lung Transplant* 2005; **24**: 1774-1780 [PMID: 16297781 DOI: 10.1016/j.healun.2005.03.017]

P- Reviewers: Cantarovich F, Cooper DKC, Fourtounas C, Holan V, Pauliks L **S- Editor**: Gou SX **L- Editor**: A **E- Editor**: Zheng XM

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.91

World J Transplant 2013 December 24; 3(4): 91-98
ISSN 2220-3230 (online)

REVIEW

Role of IL-10 in the progression of kidney disease

Inna Sinuani, Ilia Beberashvili, Zhan Averbukh, Judith Sandbank

Inna Sinuani, Judith Sandbank, Pathology Department, Assaf Harofeh Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Zerifin, 70300, Israel
Ilia Beberashvili, Zhan Averbukh, Nephrology Division, Assaf Harofeh Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Zerifin, 70300, Israel
Author contributions: Sinuani I, Sandbank J and Beberashvili I designed the research, analyzed the data and performed the search of the literature; Sinuani I wrote the paper; Averbukh Z critically revised the paper.
Correspondence to: Inna Sinuani, PhD, Pathology Department, Assaf Harofeh Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Haim Levanon 55, Zerifin 70300, Israel. sinuanii@asaf.health.gov.il
Telephone: +972-8-9779800 Fax: +972-8-9779801
Received: May 23, 2013 Revised: September 22, 2013
Accepted: October 11, 2013
Published online: December 24, 2013

Abstract

Interleukin-10 (IL-10), a cytokine with anti-inflammatory and immunomodulatory functions, regulates the biology of B and T cells. The present review describes the role of IL-10 in normal renal physiology, during acute kidney injury and in the development of chronic renal failure. We further discuss IL-10-induced cellular and molecular pathways and their link to the progression of kidney injury.

Key words: Transforming growth factor-β; Cystatin C; Interleukin-10 receptor; End-stage renal disease; Mesangial cell proliferation; Epithelial-to-mesenchymal transition; Allograft rejection

Core tip: Interleukin-10 (*IL-10*) gene expression and IL-10-induced signaling pathways have an important role in the regulation and maintenance of normal renal function. Accumulating evidence further demonstrates that abnormal IL-10 expression whether transient or prolonged, as well as interactions with other growth factors as a response to diverse stimuli is linked to the appearance and progression of a variety of kidney disorders. It has been thus suggested that selective targeting of IL-10 expression and IL-10-related pathways may provide the therapeutic features to many kidney diseases.

Sinuani I, Beberashvili I, Averbukh Z, Sandbank J. Role of IL-10 in the progression of kidney disease. *World J Transplant* 2013; 3(4): 91-98 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/91.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.91

IMMUNOLOGICAL PROPERTIES OF INTERLEUKIN-10

The anti-inflammatory Th2 cytokine interleukin-10 (IL-10) was discovered by Fiorentino and colleagues in 1989 for its ability to inhibit the synthesis of IL-2 and interferon-γ (IFN-γ) by Th1 cells[1]. To date, the IL-10 cytokine family includes nine members produced by cells, IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B and IL-29, and four viral homologs. IL-10 is produced by several T-cell subpopulations, such as Th2 and T-regulatory cells (Tregs), NK cells, and a variety of cell types, including macrophages, dendritic cells and B cells. In the kidneys, IL-10 is secreted primarily by the mesangial and endothelial cells. The viral homologs of IL-10 can be produced by Epstein-Barr virus, cytomegalovirus, ORF virus and Herpes type 2 viruses[2-4].

The gene encoding human IL-10 (5.1 kb pairs) is located on chromosome 1 and comprises five exons. The IL-10 promoter region contains several single nucleotide polymorphisms (SNPs) that influence IL-10 expression and function[5,6] and are associated with a number of diseases. Indeed, the -1082G/A SNP of the IL-10 gene is more frequent in patients with IgA nephropathy and focal segmental glomerulosclerosis and is associated with a worse prognosis of the disease[7]. The -1082G/A,

-819C/T, and -592C/A SNPs of the IL-10 promoter are consistently associated with type 2 diabetes[8], while the -1087G/A, -824C/T, -597C/A SNPs influence the prevalence of vascular-related damage in patients suffering from type 2 diabetes[9] and end-stage renal disease[10]. The -1082 SNP of the IL-10 gene affects the tumor development of renal cell carcinoma and shows a significant correlation with negative prognostic markers, such as tumor size, advanced disease stage and the presence of adenopathy[11,12].

Human IL-10 protein is a 35 kDa homodimer that is assembled from two non-covalently bound monomers. IL-10 acts through a specific receptor complex that consists of two subunits: IL-10R1 and IL-10R2. Binding of IL-10 to its receptor is a multistep process in which IL-10 initially binds to IL-10R1; the IL-10/IL-10R1 complex then binds to IL-10R2. Formation of the IL-10/IL-10R1 complex leads to modification of the cytokine's conformation, enabling presentation of the binding site to IL-10R2[13]. While the IL-10R1 subunit is highly specific for initiating IL-10 effectors functions, the IL-10R2 subunit might bind other ligands, such as TNF-α and IFN-γ. Moreover, IL-10R2 is widely present in cells that do not express IL-10R1 and are thus unresponsive to IL-10[14-17].

Activation of the IL-10 receptor complex initiates a cascade of intracellular events. The first step involves activation of members of the Janus kinase family, Jak1 and Tyk2. Activation of Jak1 is related to IL-10R1, whereas Tyk2 binds to the IL-10R2 subunit. This step is followed by activation of members of the signal transducer and activator of transcription (STAT) family. STAT1, STAT3, and STAT5 molecules in their homo- or hetero-dimeric forms enter the nucleus and bind to STAT-binding elements (SBE) in the promoters of various IL-10-responsive genes. These events enhance the transcription of anti-apoptotic genes and genes associated with cell cycle-progression, such as Bcl, Cyclin D1, Cyclin D2, Cyclin D3, Cyclin A, c-Myc, p19Ink and others[18-20]. IL-10 also induces activation of phosphatidylinositol 3-kinase and its downstream targets: p70 S6-kinase and Akt/protein kinase B. This pathway is required for the proliferative effect of IL-10[21,22]. In addition, the IL-10 signaling cascade often interacts with other intracellular pathways.

For example, IL-10 modulates the translation of TNF-α mRNA *via* the activation of p38MAPK, thereby increasing TNF-α production by mononuclear cells[23].

In human monocytes, IL-10 up-regulates the expression and activity of the general cell protective stress protein heme oxygenase-1[24].

The complexity of IL-10 activities defines a broad spectrum of the properties of IL-10. The principal function of IL-10 is to control inflammation and instruct adaptive immune responses. IL-10 inhibits the activation and differentiation of antigen-presenting cells, such as dendritic cells and macrophages. IL-10 down-regulates the expression of major histocompatibility complex class Ⅱ and co-stimulatory B7-1/B7-2 molecules and decreases the secretion of pro-inflammatory cytokines, such as TNF-α, IL-12, IL-1β, and others. IL-10 also regulates the growth and/or differentiation of B cells, NK cells, cytotoxic T and T helper cells, mast cells, keratinocytes, and endothelial and mesangial cells[2,5,25-28]. IL-10 protects the host from a variety of bacterial, parasitic, viral or fungal pathogens. Moreover, IL-10 has clear immunomodulatory properties[29-31].

IL-10 IN THE KIDNEY

IL-10 plays an important role in normal renal physiology, as well as during acute kidney injury, and in the progression of chronic renal failure.

Mesangial cells are the major local source of IL-10 in the normal adult kidney[32]. Mesangial cells are the key regulators of kidney function as they (1) provide structural support to the glomerulus by the secretion and maintenance of the extracellular matrix; (2) modulate the size of the glomerular capillary loops, thereby influencing the glomerular filtration rate; and (3) serve as both a source and target for many growth factors[33,34]. In the healthy adult kidney, mesangial cell turnover is always under tight control. Following a variety of initial insults, mesangial cells undergo activation and/or proliferation.

Activated/proliferating mesangial cells begin to secrete excessive amounts of vasoactive hormones, growth factors, cytokines, chemokines and extracellular matrix proteins. These factors in turn affect mesangial cells in an autocrine manner and mediate interactions with endothelial and epithelial tubular cells and blood-borne inflammatory cells[33,35,36]. IL-10 is an autocrine mesangial cell growth factor. *In vitro*, IL-10 induces dose-dependent proliferation of growth-arrested mesangial cells. *In vivo*, IL-10 administration to normal rats results in an increased number of intraglomerular cells and a transient reduction of creatinine clearance[28]. Several studies have demonstrated the association between the up-regulation of IL-10 and the pathophysiology of various kidney diseases, such as mesangioproliferative glomerulonephritis, IgA nephropathy, and the acute phase of microscopic polyangiitis, all of which are related to mesangial cell proliferation[37-39]. Abnormal production of growth factors by activated/proliferating mesangial cells contributes to the induction of renal structural intraglomerular and tubulointerstitial changes. These changes include glomerular and tubular cell hypertrophy, thickening of the glomerular basement membrane, and development of microalbuminuria, followed by accumulation of mesangial matrix and overt proteinuria. The degree of proteinuria correlates with the progression of glomerulosclerosis and tubulointerstitial fibrosis, pathological changes that lead to renal failure and end-stage renal disease[40]. In addition, IL-10 can promote mesangial deposition of immune complexes, thereby contributing to the progression of glomerular injury[41].

Elevated circulating IL-10 levels were found in diabetic patients. Moreover, increased IL-10 concentrations in serum predict albuminuria and correlate with the severity of diabetic nephropathy[42]. *In vivo* inhibition of IL-10 in rats with Thy1-induced glomerulonephritis greatly de-

creases glomerular mesangial cell expansion and protein excretion[43]. Anti-IL-10 treatment of mice that spontaneously develop systemic lupus erythematosus (SLE) or mice injected with peripheral blood mononuclear cells from human SLE patients delays the appearance of autoimmune manifestations. These benefits include a reduction of immune complex deposition in the glomeruli, the prevention of glomerular hypercellularity and mesangial expansion, and decreased proteinuria[44]. However, studies have shown that IL-10 is protective against SLE-induced renal damage due to the down-regulation of pathogenic Th1 responses[45]. IL-10 has a protective effect in anti-mouse glomerular basement membrane globulin-induced experimental crescentic glomerulonephritis, and the inhibition of IL-10 decreases renal function and is associated with worsening of histological features[46]. Experimental rats with chronically increased IL-10 levels after 5/6 nephrectomy show suppressed infiltration of inflammatory cells, decreased production of monocyte chemoattractant protein-1 and RANTES, and a significant reduction in mRNA for collagen type I and III in the remnant kidney. These phenomena result in a lower degree of proteinuria and a significant reduction in glomerulosclerosis and interstitial fibrosis[47]. Taken together, these findings demonstrate that under some conditions, IL-10 has a protective effect, reducing kidney injury, but in other cases, IL-10 aggravates defects in renal function. We suggest that the interdependence of the actions of IL-10 with those of other cytokines and growth factors is likely the reason for this phenomenon.

IL-10 controls the synthesis and secretion of Cystatin C (Cyst C), a cysteine protease inhibitor of great clinical importance[48]. Cyst C inhibits cathepsins and may thereby function as a tumor suppressor by inhibiting cathepsin-mediated tumor cell invasion. In addition, Cyst C regulates tissue inflammation, antigen presentation, and resistance to viral and bacterial infections[49-51]. In humans, Cyst C is produced by all nucleated cells. The blood concentrations of Cyst C are tightly correlated with the progression of autoimmune disease, inflammatory lung disorders and cardiovascular disease and may be used as a prognostic factor in cancer[51-54]. Serum Cyst C levels may be more accurate than the glomerular filtration rate as diagnostic value of renal function[55,56]. Today, the concentrations of Cyst C in serum and urine are used as reliable markers of acute kidney injury[57,58]. Similar to IL-10, Cyst C induces mesangial cell proliferation in an autocrine manner[59].

Another growth factor whose functions are closely related to IL-10 is transforming growth factor-β (TGF-β). The TGF-β-induced signaling network plays an important role in human diseases. TGF-β has an essential role in both normal kidney function and during the progression of renal injury. TGF-β executes its actions through activation of the Smad and mitogen-activated protein kinase intracellular signaling pathways. TGF-β isoforms are widely present and act on virtually every cell type. TGF-β regulates the proliferation, differentiation, migration, hypertrophy and apoptosis of intraglomerular and tubular cells, controls remodeling of the extracellular matrix, and promotes glomerular and interstitial fibrosis and the progression of glomerulosclerosis[60-64].

Furthermore, TGF-β induces the process of epithelial-to-mesenchymal transition (EMT) in normal mammary epithelial cells. During EMT, cells lose their epithelial identity, reflected in the loss of the expression of proteins associated with epithelial morphology, such as E-cadherin, α-, β-, and γ-catenins, and zonula occludens-1, and begin to synthesize *de novo* proteins associated with a mesenchymal phenotype, such as D-cadherin, fibronectin, vimentin, and α-smooth muscle actin. These events occur in parallel with a decrease in cell-cell adhesion and changes in the actin cytoskeleton[65,66]. TGF-β-induced EMT in podocytes is responsible for the appearance and progression of albuminuria and proteinuria. The severity of proteinuria correlates with the progression of glomerulosclerosis and tubulointerstitial fibrosis. Fibrosis is usually preceded by the infiltration of mononuclear inflammatory cells into the interstitium. These cells secrete cytokines and chemokines that stimulate resident tubular epithelial cells to differentiate into matrix-producing fibroblasts[64,67-69]. IL-10 and TGF-β may act synergistically to regulate the production of proinflammatory cytokines, chemokines and nitric oxide by mononuclear cells. Moreover, TGF-β induces IL-10 expression and vice versa in various cell types, including mesangial cells[7,32,70,71].

IL-10 acts on both TGF-β and Cyst C, and TGF-β and Cyst C separately influence IL-10 synthesis and activity[70,71-73]. It has also been shown that there is direct cross talk between TGF-β and Cyst C. TGF-β induces Cyst C expression[49,74], while Cyst C acts as a TGF-β antagonist that prevents the binding of TGF-β to its receptor and thereby inhibits its activity[49,75]. Cyst C is important in the acute phase of the kidney's responses to injury, which are rapid and aggressive, whereas TGF-β promotes slower processes that lead to chronic renal failure and end-stage renal disease. It has been suggested that the major role of the dialogue between IL-10 and TGF-β, IL-10 and Cyst C, and Cyst C and TGF-β is to instruct and regulate the degree of the renal responses to primary injury. These responses include control of mesangial cell proliferation, accumulation of extracellular matrix, influx of mononuclear cells, glomerulosclerosis, and tubular fibrosis (Figure 1).

IL-10 IN ALLOGRAFT SURVIVAL/ REJECTION

Transplantation has become an accepted treatment for end-stage renal disease. The major barrier of transplantation from genetically disparate donors is the process of rejection, in which the recipient's immune system recognizes the graft as foreign tissue and attacks it. Allograft rejection can occur through direct (cellular) or indirect (humoral) pathways and is a complex process involving both cell-mediated immunity and circulating antibodies. The role of cytokines and the particular role of IL-10 and the IL-10-induced signaling network in the develop-

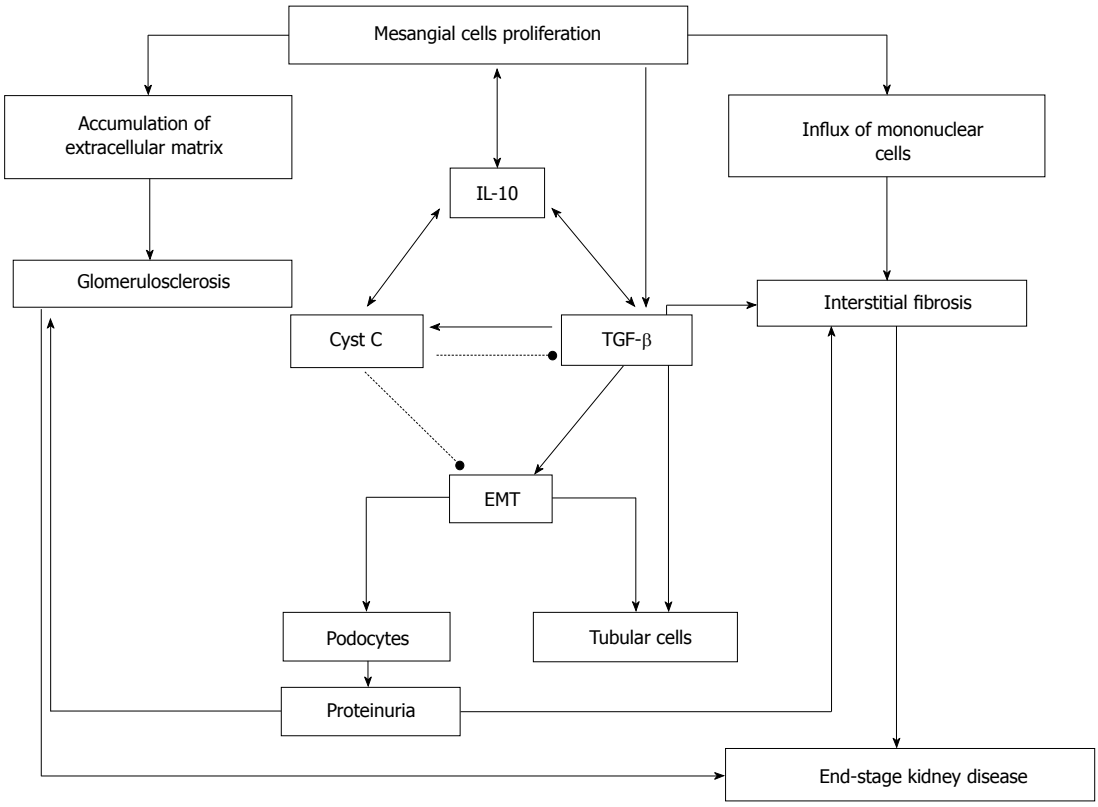

Figure 1 Interleukin-10 functions in the progression of renal failure. Interleukin-10 (IL-10) induces over-proliferation of mesangial cells that through an increased synthesis and secretion of a variety of growth factors, cytokines and chemokines, evoke several pathologic processes, leading to progression of renal failure. An increased secretion of components comprising the mesangial extracellular matrix results in its accumulation and is followed by the formation of fibrotic and sclerotic lesions in the glomeruli. IL-10 induces the synthesis and activity of Cystatin C (Cyst C) and transforming growth factor-β (TGF-β). Cystatin C regulates tissue inflammation and increases mesangial cell proliferation. Increased TGF-β levels act in parallel with IL-10 to promote fibrosis and glomerulosclerosis. In addition, the TGF-β-induced epithelial-to-mesenchymal transition in podocytes leads to the appearance of proteinuria. The development of proteinuria aggravates the processes of glomerulosclerosis and interstitial fibrosis and leads to end-stage kidney disease. EMT: Epithelial-to-mesenchymal transition.

ment and progression of graft survival/rejection are subjects of intensive research[76-80]. Indeed, the IL-10-TGF-β pathway plays an important role in the progression of allograft fibrosis, while TGF-β is a potential therapeutic target for the prevention and therapy of fibrogenesis in kidney transplants[76,81]. However, whether IL-10 plays an overall helpful or detrimental role is not yet known. High intra-graft IL-10 expression was found in patients undergoing acute rejection[82]. In the case of chronic rejection, poorer graft survival, which is generally associated with evidence of interstitial fibrosis and tubular atrophy, is accompanied by the up-regulation of IL-10 gene expression[83]. IL-10 is also a stimulator of the immune system, inducing the differentiation and proliferation of B cells, thus leading the immune response toward the humoral pathway and enhancing antibody responses against the graft[83,84]. In contrast, IL-10 has a clear protective effect. It has been shown that the up-regulation of the IL-10 gene in a rat model of kidney allograft rejection improves renal function and prolongs allograft survival[85]. IL-10, when secreted by T-regulatory cells, suppresses antigen-specific effector cell responses *via* inhibition of pro-inflammatory cytokine production[86]. Additional findings show that an acute immune response during graft rejection is associated with an over-expression of pro-inflammatory Th1 cytokines, which appear in parallel with the accumulation of IL-10. It has been suggested that in this situation, the rise in IL-10 levels serves to regulate and limit the inflammatory responses[87].

IL-10 IN COMPENSATORY RENAL GROWTH

The discovery of the compensatory renal growth process is, without a doubt, the most important reason why the expansion of kidney transplantation from live donors has occurred in recent years. After removal of a single kidney, the remaining kidney becomes enlarged, mainly through the hypertrophy of tubular cells, and compensates for the loss of the contralateral organ within a short period of time. TGF-β has been suggested as the most important factor causing tubular cell hypertrophy and therefore has a pivotal role in compensatory renal growth[88]. Although the tubular cells are the main site at which compensatory renal growth takes place, studies from our group showed that mesangial cells initiate compensatory renal growth and control the degree of compensatory tubular cell hypertrophy by controlling IL-10 to TGF-β cross-talk[32,89].

Immediately after unilateral nephrectomy, the remaining kidney undergoes hyperfiltration. The changes in

glomerular hemodynamics lead to a transient proliferation of mesangial cells, reaching a maximum at 24 h after surgery; proliferation is then arrested within 72 h. Proliferating mesangial cells secrete increased amounts of many growth factors, including IL-10. These growth factors affect mesangial cells in an autocrine manner as additional stimuli to over-proliferate, influence the conversion of TGF-β from the latent to the active form, and lead to increased TGF-β production. Among the resident renal cell types studied, only mesangial cells secrete and activate TGF-β[90,91]. A reduction in mesangial cell proliferation occurs in parallel with the appearance of renal tubular cell hypertrophy. When TGF-β accumulates to sufficient levels, it induces tubular cells to undergo hypertrophy themselves and, in parallel, acts on mesangial cells to inhibit their proliferation. Inhibition of mesangial cell proliferation, in turn, reduces TGF-β levels and inhibits compensatory tubular cell hypertrophy. TGF-β secretion may be affected by many growth factors, including angiotensin II, IGF-I, HGF, bFGF, TNF-α, EGF, PDGF, and others, all of which are produced by the mesangial cells[92-95]. The importance of IL-10 in this process may be underscored by the fact that the *in vivo* inhibition of IL-10 production by mesangial cells leads to a significant reduction in TGF-β expression in the remaining kidney; this is accompanied by an approximate 25% reduction in remaining kidney weight and a significant decrease in compensatory tubular cell hypertrophy[32,89]. Compensatory renal growth is regulated by a variety of growth factors and cytokines that initiate proliferative, hypertrophic, and apoptotic growth responses in the remaining kidneys. These growth factors may act in concert, and despite their apparent redundancy, they all must be present in sufficient concentrations to support maximal growth of the remaining kidney. Due to the interdependence between these cytokines, manipulation of the expression of one of these may affect the entire compensatory growth response in the remaining kidney.

In summary, IL-10 gene expression and IL-10-induced signaling pathways have an important role in the regulation and maintenance of normal renal function. Moreover, accumulating evidence further demonstrates that abnormal IL-10 expression, whether transient or prolonged, as well as interactions with other growth factors as a response to diverse stimuli, is linked to the appearance and progression of a variety of kidney disorders. It has thus been suggested that the selective targeting of IL-10 expression and IL-10-related pathways may provide therapeutic approaches for many kidney diseases.

REFERENCES

1 **Fiorentino DF**, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989; **170**: 2081-2095 [PMID: 2531194]

2 **Moore KW**, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; **19**: 683-765 [PMID: 11244051]

3 **Sabat R**. IL-10 family of cytokines. *Cytokine Growth Factor Rev* 2010; **21**: 315-324 [PMID: 21112807 DOI: 10.1016/j.cytogfr.2010.11.001]

4 **Zdanov A**. Structural analysis of cytokines comprising the IL-10 family. *Cytokine Growth Factor Rev* 2010; **21**: 325-330 [PMID: 20846897 DOI: 10.1016/j.cytogfr.2010.08.003]

5 **Sabat R**, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. Biology of interleukin-10. *Cytokine Growth Factor Rev* 2010; **21**: 331-344 [PMID: 21115385 DOI: 10.1016/j.cytogfr.2010.09.002]

6 **Kim JM**, Brannan CI, Copeland NG, Jenkins NA, Khan TA, Moore KW. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. *J Immunol* 1992; **148**: 3618-3623 [PMID: 1350294]

7 **Bantis C**, Heering PJ, Aker S, Klein-Vehne N, Grabensee B, Ivens K. Association of interleukin-10 gene G-1082A polymorphism with the progression of primary glomerulonephritis. *Kidney Int* 2004; **66**: 288-294 [PMID: 15200436]

8 **Mtiraoui N**, Ezzidi I, Kacem M, Ben Hadj Mohamed M, Chaieb M, Haj Jilani AB, Mahjoub T, Almawi WY. Predictive value of interleukin-10 promoter genotypes and haplotypes in determining the susceptibility to nephropathy in type 2 diabetes patients. *Diabetes Metab Res Rev* 2009; **25**: 57-63 [PMID: 19031431 DOI: 10.1002/dmrr.892]

9 **Forte GI**, Pilato G, Vaccarino L, Sanacore M, Candore G, Romano GC, Testa R, Franceschi C, Capri M, Marra M, Bonfigli AR, Caruso C, Scola L, Lio D. Risk profiles in type 2 diabetes (metabolic syndrome): integration of IL-10 polymorphisms and laboratory parameters to identify vascular damages related complications. *Curr Pharm Des* 2010; **16**: 898-903 [PMID: 20388104]

10 **Nordfors L**, Lindholm B, Stenvinkel P. End-stage renal disease--not an equal opportunity disease: the role of genetic polymorphisms. *J Intern Med* 2005; **258**: 1-12 [PMID: 15953127]

11 **Romero JM**, Sáenz-López P, Cózar JM, Carretero R, Canton J, Vazquez F, Concha A, Tallada M, Garrido F, Ruiz-Cabello F. A polymorphism in the interleukin-10 promoter affects the course of disease in patients with clear-cell renal carcinoma. *Hum Immunol* 2009; **70**: 60-64 [PMID: 19027043 DOI: 10.1016/j.humimm.2008.10.020]

12 **Havranek E**, Howell WM, Fussell HM, Whelan JA, Whelan MA, Pandha HS. An interleukin-10 promoter polymorphism may influence tumor development in renal cell carcinoma. *J Urol* 2005; **173**: 709-712 [PMID: 15711248]

13 **Yoon SI**, Logsdon NJ, Sheikh F, Donnelly RP, Walter MR. Conformational changes mediate interleukin-10 receptor 2 (IL-10R2) binding to IL-10 and assembly of the signaling complex. *J Biol Chem* 2006; **281**: 35088-35096 [PMID: 16982608]

14 **Yoon SI**, Jones BC, Logsdon NJ, Harris BD, Deshpande A, Radaeva S, Halloran BA, Gao B, Walter MR. Structure and mechanism of receptor sharing by the IL-10R2 common chain. *Structure* 2010; **18**: 638-648 [PMID: 20462497 DOI: 10.1016/j.str.2010.02.009]

15 **Ding Y**, Qin L, Zamarin D, Kotenko SV, Pestka S, Moore KW, Bromberg JS. Differential IL-10R1 expression plays a critical role in IL-10-mediated immune regulation. *J Immunol* 2001; **167**: 6884-6892 [PMID: 11739506]

16 **Kotenko SV**, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol* 2003; **4**: 69-77 [PMID: 12483210]

17 **Xie MH**, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD, Gurney AL. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem* 2000; **275**: 31335-31339 [PMID: 10875937]

18 **Finbloom DS**, Winestock KD. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential as-

sembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. *J Immunol* 1995; **155**: 1079-1090 [PMID: 7543512]
19 **Wehinger J**, Gouilleux F, Groner B, Finke J, Mertelsmann R, Weber-Nordt RM. IL-10 induces DNA binding activity of three STAT proteins (Stat1, Stat3, and Stat5) and their distinct combinatorial assembly in the promoters of selected genes. *FEBS Lett* 1996; **394**: 365-370 [PMID: 8830676]
20 **Donnelly RP**, Dickensheets H, Finbloom DS. The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. *J Interferon Cytokine Res* 1999; **19**: 563-573 [PMID: 10433356]
21 **Crawley JB**, Williams LM, Mander T, Brennan FM, Foxwell BM. Interleukin-10 stimulation of phosphatidylinositol 3-kinase and p70 S6 kinase is required for the proliferative but not the antiinflammatory effects of the cytokine. *J Biol Chem* 1996; **271**: 16357-16362 [PMID: 8663063]
22 **Strle K**, Zhou JH, Broussard SR, Venters HD, Johnson RW, Freund GG, Dantzer R, Kelley KW. IL-10 promotes survival of microglia without activating Akt. *J Neuroimmunol* 2002; **122**: 9-19 [PMID: 11777539]
23 **Kontoyiannis D**, Kotlyarov A, Carballo E, Alexopoulou L, Blackshear PJ, Gaestel M, Davis R, Flavell R, Kollias G. Interleukin-10 targets p38 MAPK to modulate ARE-dependent TNF mRNA translation and limit intestinal pathology. *EMBO J* 2001; **20**: 3760-3770 [PMID: 11447117]
24 **Jung M**, Sabat R, Krätzschmar J, Seidel H, Wolk K, Schönbein C, Schütt S, Friedrich M, Döcke WD, Asadullah K, Volk HD, Grütz G. Expression profiling of IL-10-regulated genes in human monocytes and peripheral blood mononuclear cells from psoriatic patients during IL-10 therapy. *Eur J Immunol* 2004; **34**: 481-493 [PMID: 14768053]
25 **Mosmann TR**. Role of a new cytokine, interleukin-10, in the cross-regulation of T helper cells. *Ann N Y Acad Sci* 1991; **628**: 337-344 [PMID: 1648883]
26 **Hedrich CM**, Bream JH. Cell type-specific regulation of IL-10 expression in inflammation and disease. *Immunol Res* 2010; **47**: 185-206 [PMID: 20087682 DOI: 10.1007/s12026-009-8150-5]
27 **Fiorentino DF**, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; **147**: 3815-3822 [PMID: 1940369]
28 **Chadban SJ**, Tesch GH, Foti R, Atkins RC, Nikolic-Paterson DJ. Interleukin-10 is a mesangial cell growth factor in vitro and in vivo. *Lab Invest* 1997; **76**: 619-627 [PMID: 9166281]
29 **Saraiva M**, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010; **10**: 170-181 [PMID: 20154735 DOI: 10.1038/nri2711]
30 **Couper KN**, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008; **180**: 5771-5777 [PMID: 18424693]
31 **Strassmann G**, Kambayashi T, Jacob CO, Sredni D. The immunomodulator AS-101 inhibits IL-10 release and augments TNF alpha and IL-1 alpha release by mouse and human mononuclear phagocytes. *Cell Immunol* 1997; **176**: 180-185 [PMID: 9073392]
32 **Sinuani I**, Averbukh Z, Gitelman I, Rapoport MJ, Sandbank J, Albeck M, Sredni B, Weissgarten J. Mesangial cells initiate compensatory renal tubular hypertrophy via IL-10-induced TGF-beta secretion: effect of the immunomodulator AS101 on this process. *Am J Physiol Renal Physiol* 2006; **291**: F384-F394 [PMID: 16571592]
33 **Cove-Smith A**, Hendry BM. The regulation of mesangial cell proliferation. *Nephron Exp Nephrol* 2008; **108**: e74-e79 [PMID: 18431073 DOI: 10.1159/000127359]
34 **Stockand JD**, Sansom SC. Glomerular mesangial cells: electrophysiology and regulation of contraction. *Physiol Rev* 1998; **78**: 723-744 [PMID: 9674692]
35 **Schena FP**, Strippoli GF, Wankelmuth P. Renal growth factors: past, present and future. *Am J Nephrol* 1999; **19**: 308-312 [PMID: 10213833]
36 **Schlöndorff D**, Banas B. The mesangial cell revisited: no cell is an island. *J Am Soc Nephrol* 2009; **20**: 1179-1187 [PMID: 19470685 DOI: 10.1681/ASN.2008050549]
37 **Yano N**, Endoh M, Nomoto Y, Sakai H, Fadden K, Rifai A. Phenotypic characterization of cytokine expression in patients with IgA nephropathy. *J Clin Immunol* 1997; **17**: 396-403 [PMID: 9327339]
38 **Niemir ZI**, Ondracek M, Dworacki G, Stein H, Waldherr R, Ritz E, Otto HF. In situ upregulation of IL-10 reflects the activity of human glomerulonephritides. *Am J Kidney Dis* 1998; **32**: 80-92 [PMID: 9669428]
39 **Hirschberg R**, Adler S. Insulin-like growth factor system and the kidney: physiology, pathophysiology, and therapeutic implications. *Am J Kidney Dis* 1998; **31**: 901-919 [PMID: 9631833]
40 **Makino H**, Kashihara N, Sugiyama H, Kanao K, Sekikawa T, Okamoto K, Maeshima Y, Ota Z, Nagai R. Phenotypic modulation of the mesangium reflected by contractile proteins in diabetes. *Diabetes* 1996; **45**: 488-495 [PMID: 8603771]
41 **Lakkis FG**, Baddoura FK, Cruet EN, Parekh KR, Fukunaga M, Munger KA. Anti-inflammatory lymphokine mRNA expression in antibody-induced glomerulonephritis. *Kidney Int* 1996; **49**: 117-126 [PMID: 8770957]
42 **Myśliwska J**, Zorena K, Semetkowska-Jurkiewicz E, Rachoń D, Suchanek H, Myśliwski A. High levels of circulating interleukin-10 in diabetic nephropathy patients. *Eur Cytokine Netw* 2005; **16**: 117-122 [PMID: 15941682]
43 **Kalechman Y**, Gafter U, Weinstein T, Chagnac A, Freidkin I, Tobar A, Albeck M, Sredni B. Inhibition of interleukin-10 by the immunomodulator AS101 reduces mesangial cell proliferation in experimental mesangioproliferative glomerulonephritis: association with dephosphorylation of STAT3. *J Biol Chem* 2004; **279**: 24724-24732 [PMID: 15001575]
44 **Kalechman Y**, Gafter U, Gal R, Rushkin G, Yan D, Albeck M, Sredni B. Anti-IL-10 therapeutic strategy using the immunomodulator AS101 in protecting mice from sepsis-induced death: dependence on timing of immunomodulating intervention. *J Immunol* 2002; **169**: 384-392 [PMID: 12077268]
45 **Ling GS**, Cook HT, Botto M, Lau YL, Huang FP. An essential protective role of IL-10 in the immunological mechanism underlying resistance vs. susceptibility to lupus induction by dendritic cells and dying cells. *Rheumatology (Oxford)* 2011; **50**: 1773-1784 [PMID: 21727182 DOI: 10.1093/rheumatology/ker198]
46 **Summers SA**, Phoon RK, Odobasic D, Dewage L, Kitching AR, Holdsworth SR. Signal transducer and activation of transcription 6 (STAT6) regulates T helper type 1 (Th1) and Th17 nephritogenic immunity in experimental crescentic glomerulonephritis. *Clin Exp Immunol* 2011; **166**: 227-234 [PMID: 21985369 DOI: 10.1111/j.1365-2249.2011.04437.x]
47 **Mu W**, Ouyang X, Agarwal A, Zhang L, Long DA, Cruz PE, Roncal CA, Glushakova OY, Chiodo VA, Atkinson MA, Hauswirth WW, Flotte TR, Rodriguez-Iturbe B, Johnson RJ. IL-10 suppresses chemokines, inflammation, and fibrosis in a model of chronic renal disease. *J Am Soc Nephrol* 2005; **16**: 3651-3660 [PMID: 16251240]
48 **Xu Y**, Schnorrer P, Proietto A, Kowalski G, Febbraio MA, Acha-Orbea H, Dickins RA, Villadangos JA. IL-10 controls cystatin C synthesis and blood concentration in response to inflammation through regulation of IFN regulatory factor 8 expression. *J Immunol* 2011; **186**: 3666-3673 [PMID: 21300820 DOI: 10.4049/jimmunol.1001934]
49 **Vray B**, Hartmann S, Hoebeke J. Immunomodulatory properties of cystatins. *Cell Mol Life Sci* 2002; **59**: 1503-1512 [PMID: 12440772]
50 **Sokol JP**, Schiemann WP. Cystatin C antagonizes transforming growth factor beta signaling in normal and cancer cells. *Mol Cancer Res* 2004; **2**: 183-195 [PMID: 15037657]

51 **Kos J**, Werle B, Lah T, Brunner N. Cysteine proteinases and their inhibitors in extracellular fluids: markers for diagnosis and prognosis in cancer. *Int J Biol Markers* 2000; **15**: 84-89 [PMID: 10763147]

52 **Hansen T**, Petrow PK, Gaumann A, Keyszer G, Bräuer R, Kriegsmann J. Synovial giant cells in rheumatoid arthritis: expression of cystatin C, but not of cathepsin B. *Exp Toxicol Pathol* 2000; **52**: 312-316 [PMID: 10987183]

53 **Kos J**, Krasovec M, Cimerman N, Nielsen HJ, Christensen IJ, Brünner N. Cysteine proteinase inhibitors stefin A, stefin B, and cystatin C in sera from patients with colorectal cancer: relation to prognosis. *Clin Cancer Res* 2000; **6**: 505-511 [PMID: 10690531]

54 **Koenig W**, Twardella D, Brenner H, Rothenbacher D. Plasma concentrations of cystatin C in patients with coronary heart disease and risk for secondary cardiovascular events: more than simply a marker of glomerular filtration rate. *Clin Chem* 2005; **51**: 321-327 [PMID: 15563478]

55 **Shlipak MG**. Cystatin C as a marker of glomerular filtration rate in chronic kidney disease: influence of body composition. *Nat Clin Pract Nephrol* 2007; **3**: 188-189 [PMID: 17290239]

56 **Heilman RL**, Mazur MJ. Cystatin C as a more sensitive indicator of diminished glomerular filtration rate. *Liver Transpl* 2005; **11**: 264-266 [PMID: 15719394]

57 **Zhang Z**, Lu B, Sheng X, Jin N. Cystatin C in prediction of acute kidney injury: a systemic review and meta-analysis. *Am J Kidney Dis* 2011; **58**: 356-365 [PMID: 21601330 DOI: 10.1053/j.ajkd.2011.02.389]

58 **Koyner JL**, Vaidya VS, Bennett MR, Ma Q, Worcester E, Akhter SA, Raman J, Jeevanandam V, O'Connor MF, Devarajan P, Bonventre JV, Murray PT. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol* 2010; **5**: 2154-2165 [PMID: 20798258 DOI: 10.2215/CJN.00740110]

59 **Tavéra C**, Leung-Tack J, Prévot D, Gensac MC, Martinez J, Fulcrand P, Collé A. Cystatin C secretion by rat glomerular mesangial cells: autocrine loop for in vitro growth-promoting activity. *Biochem Biophys Res Commun* 1992; **182**: 1082-1088 [PMID: 1540157]

60 **Blobe GC**, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med* 2000; **342**: 1350-1358 [PMID: 10793168]

61 **Böttinger EP**, Bitzer M. TGF-beta signaling in renal disease. *J Am Soc Nephrol* 2002; **13**: 2600-2610 [PMID: 12239251]

62 **Böttinger EP**. TGF-beta in renal injury and disease. *Semin Nephrol* 2007; **27**: 309-320 [PMID: 17533008]

63 **Marks DJ**, Segal AW. Innate immunity in inflammatory bowel disease: a disease hypothesis. *J Pathol* 2008; **214**: 260-266 [PMID: 18161747]

64 **Liu Y**. Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int* 2006; **69**: 213-217 [PMID: 16408108]

65 **Miettinen PJ**, Ebner R, Lopez AR, Derynck R. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. *J Cell Biol* 1994; **127**: 2021-2036 [PMID: 7806579]

66 **Zavadil J**, Bitzer M, Liang D, Yang YC, Massimi A, Kneitz S, Piek E, Bottinger EP. Genetic programs of epithelial cell plasticity directed by transforming growth factor-beta. *Proc Natl Acad Sci U S A* 2001; **98**: 6686-6691 [PMID: 11390996]

67 **Piek E**, Moustakas A, Kurisaki A, Heldin CH, ten Dijke P. TGF-(beta) type I receptor/ALK-5 and Smad proteins mediate epithelial to mesenchymal transdifferentiation in NMuMG breast epithelial cells. *J Cell Sci* 1999; **112** (Pt 24): 4557-4568 [PMID: 10574705]

68 **Fan JM**, Ng YY, Hill PA, Nikolic-Paterson DJ, Mu W, Atkins RC, Lan HY. Transforming growth factor-beta regulates tubular epithelial-myofibroblast transdifferentiation in vitro. *Kidney Int* 1999; **56**: 1455-1467 [PMID: 10504497]

69 **Kang YS**, Li Y, Dai C, Kiss LP, Wu C, Liu Y. Inhibition of integrin-linked kinase blocks podocyte epithelial-mesenchymal transition and ameliorates proteinuria. *Kidney Int* 2010; **78**: 363-373 [PMID: 20505657 DOI: 10.1038/ki.2010.137]

70 **Bickerstaff AA**, VanBuskirk AM, Wakely E, Orosz CG. Transforming growth factor-beta and interleukin-10 subvert alloreactive delayed type hypersensitivity in cardiac allograft acceptor mice. *Transplantation* 2000; **69**: 1517-1520 [PMID: 10798784]

71 **Maeda H**, Kuwahara H, Ichimura Y, Ohtsuki M, Kurakata S, Shiraishi A. TGF-beta enhances macrophage ability to produce IL-10 in normal and tumor-bearing mice. *J Immunol* 1995; **155**: 4926-4932 [PMID: 7594497]

72 **Fuss IJ**, Boirivant M, Lacy B, Strober W. The interrelated roles of TGF-beta and IL-10 in the regulation of experimental colitis. *J Immunol* 2002; **168**: 900-908 [PMID: 11777988]

73 **Frendéus KH**, Wallin H, Janciauskiene S, Abrahamson M. Macrophage responses to interferon-gamma are dependent on cystatin C levels. *Int J Biochem Cell Biol* 2009; **41**: 2262-2269 [PMID: 19446036 DOI: 10.1016/j.biocel.2009.05.005]

74 **Afonso S**, Tovar C, Romagnano L, Babiarz B. Control and expression of cystatin C by mouse decidual cultures. *Mol Reprod Dev* 2002; **61**: 155-163 [PMID: 11803549]

75 **Sokol JP**, Neil JR, Schiemann BJ, Schiemann WP. The use of cystatin C to inhibit epithelial-mesenchymal transition and morphological transformation stimulated by transforming growth factor-beta. *Breast Cancer Res* 2005; **7**: R844-R853 [PMID: 16168131]

76 **VanBuskirk AM**, Burlingham WJ, Jankowska-Gan E, Chin T, Kusaka S, Geissler F, Pelletier RP, Orosz CG. Human allograft acceptance is associated with immune regulation. *J Clin Invest* 2000; **106**: 145-155 [PMID: 10880058]

77 **Waaga AM**, Gasser M, Kist-van Holthe JE, Najafian N, Müller A, Vella JP, Womer KL, Chandraker A, Khoury SJ, Sayegh MH. Regulatory functions of self-restricted MHC class II allopeptide-specific Th2 clones in vivo. *J Clin Invest* 2001; **107**: 909-916 [PMID: 11285310]

78 **Keller MR**, Burlingham WJ. Loss of tolerance to self after transplant. *Semin Immunopathol* 2011; **33**: 105-110 [PMID: 21298434 DOI: 10.1007/s00281-011-0252-1]

79 **Pöge U**, Gerhardt T, Stoffel-Wagner B, Palmedo H, Klehr HU, Sauerbruch T, Woitas RP. Cystatin C-based calculation of glomerular filtration rate in kidney transplant recipients. *Kidney Int* 2006; **70**: 204-210 [PMID: 16723983]

80 **Le Bricon T**, Thervet E, Benlakehal M, Bousquet B, Legendre C, Erlich D. Changes in plasma cystatin C after renal transplantation and acute rejection in adults. *Clin Chem* 1999; **45**: 2243-2249 [PMID: 10585359]

81 **Djamali A**, Samaniego M. Fibrogenesis in kidney transplantation: potential targets for prevention and therapy. *Transplantation* 2009; **88**: 1149-1156 [PMID: 19935366 DOI: 10.1097/TP.0b013e3181bcccea]

82 **Strehlau J**, Pavlakis M, Lipman M, Shapiro M, Vasconcellos L, Harmon W, Strom TB. Quantitative detection of immune activation transcripts as a diagnostic tool in kidney transplantation. *Proc Natl Acad Sci U S A* 1997; **94**: 695-700 [PMID: 9012847]

83 **Hueso M**, Navarro E, Moreso F, O'Valle F, Pérez-Riba M, Del Moral RG, Grinyó JM, Serón D. Intragraft expression of the IL-10 gene is up-regulated in renal protocol biopsies with early interstitial fibrosis, tubular atrophy, and subclinical rejection. *Am J Pathol* 2010; **176**: 1696-1704 [PMID: 20150436 DOI: 10.2353/ajpath.2010.090411]

84 **Rousset F**, Garcia E, Defrance T, Péronne C, Vezzio N, Hsu DH, Kastelein R, Moore KW, Banchereau J. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci U S A* 1992; **89**: 1890-1893 [PMID: 1371884]

85 **Chen B**, Kapturczak MH, Joseph R, George JF, Campbell-Thompson M, Wasserfall CH, Atkinson MA, Tisher CC, Flotte TR, Agarwal A, Chen S. Adeno-associated viral

vector-mediated interleukin-10 prolongs allograft survival in a rat kidney transplantation model. *Am J Transplant* 2007; **7**: 1112-1120 [PMID: 17456199]

86 **Roncarolo MG**, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev* 2006; **212**: 28-50 [PMID: 16903904]

87 **Edemir B**, Kurian SM, Eisenacher M, Lang D, Müller-Tidow C, Gabriëls G, Salomon DR, Schlatter E. Activation of counter-regulatory mechanisms in a rat renal acute rejection model. *BMC Genomics* 2008; **9**: 71 [PMID: 18261221 DOI: 10.1186/1471-2164-9-71]

88 **Liu B**, Preisig P. TGF-beta1-mediated hypertrophy involves inhibiting pRB phosphorylation by blocking activation of cyclin E kinase. *Am J Physiol* 1999; **277**: F186-F194 [PMID: 10444572]

89 **Sinuani I**, Beberashvili I, Averbukh Z, Cohn M, Gitelman I, Weissgarten J. Mesangial cells initiate compensatory tubular cell hypertrophy. *Am J Nephrol* 2010; **31**: 326-331 [PMID: 20160436 DOI: 10.1159/000287229]

90 **Kitamura M**, Sütö T, Yokoo T, Shimizu F, Fine LG. Transforming growth factor-beta 1 is the predominant paracrine inhibitor of macrophage cytokine synthesis produced by glomerular mesangial cells. *J Immunol* 1996; **156**: 2964-2971 [PMID: 8609417]

91 **Kaname S**, Uchida S, Ogata E, Kurokawa K. Autocrine secretion of transforming growth factor-beta in cultured rat mesangial cells. *Kidney Int* 1992; **42**: 1319-1327 [PMID: 1474764]

92 **Wolf G**, Ziyadeh FN, Zahner G, Stahl RA. Angiotensin II is mitogenic for cultured rat glomerular endothelial cells. *Hypertension* 1996; **27**: 897-905 [PMID: 8613266]

93 **Song K**, Cornelius SC, Reiss M, Danielpour D. Insulin-like growth factor-I inhibits transcriptional responses of transforming growth factor-beta by phosphatidylinositol 3-kinase/Akt-dependent suppression of the activation of Smad3 but not Smad2. *J Biol Chem* 2003; **278**: 38342-38351 [PMID: 12876289]

94 **Sullivan DE**, Ferris M, Pociask D, Brody AR. The latent form of TGFbeta(1) is induced by TNFalpha through an ERK specific pathway and is activated by asbestos-derived reactive oxygen species in vitro and in vivo. *J Immunotoxicol* 2008; **5**: 145-149 [PMID: 18569384 DOI: 10.1080/15476910802085822]

95 **Hardie WD**, Davidson C, Ikegami M, Leikauf GD, Le Cras TD, Prestridge A, Whitsett JA, Korfhagen TR. EGF receptor tyrosine kinase inhibitors diminish transforming growth factor-alpha-induced pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008; **294**: L1217-L1225 [PMID: 18424623 DOI: 10.1152/ajplung.00020.2008]

P- Reviewer: Scherer A **S- Editor**: Wen LL **L- Editor**: A
E- Editor: Yan JL

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.99

World J Transplant 2013 December 24; 3(4): 99-112
ISSN 2220-3230 (online)

REVIEW

Optimal stem cell source for allogeneic stem cell transplantation for hematological malignancies

Daniel KL Cheuk

Daniel KL Cheuk, Department of Paediatrics and Adolescent Medicine, the University of Hong Kong, Queen Mary Hospital, Hong Kong, China
Author contributions: Cheuk DKL solely contributed to this paper.
Supported by The University of Hong Kong
Correspondence to: Daniel KL Cheuk, FHKAM, Department of Paediatrics and Adolescent Medicine, the University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong, China. cheukkld@hkucc.hku.hk
Telephone: +86-852-22553909 Fax: +86-852-22551523
Received: August 28, 2013 Revised: November 15, 2013
Accepted: December 9, 2013
Published online: December 24, 2013

Abstract

Hematopoietic stem cell transplant (HSCT) is a standard treatment for many hematological malignancies. Three different sources of stem cells, namely bone marrow (BM), peripheral blood stem cells (PBSC) and cord blood (CB) can be used for HSCT, and each has its own advantages and disadvantages. Randomized controlled trials (RCTs) suggest that there is no significant survival advantage of PBSC over BM in Human Leukocyte Antigen-matched sibling transplant for adult patients with hematological malignancies. PBSC transplant probably results in lower risk of relapse and hence better disease-free survival, especially in patients with high risk disease at the expense of higher risks of both severe acute and chronic graft-versus-host disease (GVHD). In the unrelated donor setting, the only RCT available suggests that PBSC and BM result in comparable overall and disease-free survivals in patients with hematological malignancies; and PBSC transplant results in lower risk of graft failure and higher risk of chronic GVHD. High level evidence is not available for CB in comparison to BM or PBSC. The risks and benefits of different sources of stem cells likely change with different conditioning regimen, strategies for prophylaxis and treatment of GVHD and manipulation of grafts. The recent success and rapid advance of double CB transplant and haploidentical BM and PBSC transplants further complicate the selection of stem cell source. Optimal selection requires careful weighing of the risks and benefits of different stem cell source for each individual recipient and donor. Detailed counseling of patient and donor regarding risks and benefits in the specific context of the patient and transplant method is essential for informed decision making.

Key words: Hematopoietic stem cell transplantation; Bone marrow; Peripheral blood stem cell; Cord blood; Hematological malignancy

Core tip: Randomized controlled trials (RCTs) suggest no difference in survival between peripheral blood stem cell (PBSC) and bone marrow (BM) in matched sibling transplant for patients with hematological malignancies. PBSC may result in fewer relapse in high risk patients but more severe graft-versus-host disease (GVHD). For unrelated donor, the only RCT suggests PBSC and BM result in comparable survivals, with PBSC resulting in fewer graft failure but more chronic GVHD. RCT is not available to compare cord blood with BM or PBSC. The risks and benefits of different sources of stem cells likely change with transplant methods and manipulation of grafts.

Cheuk DKL. Optimal stem cell source for allogeneic stem cell transplantation for hematological malignancies. *World J Transplant* 2013; 3(4): 99-112 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/99.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.99

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is now

Table 1 Comparison of bone marrow, peripheral blood stem cell and cord blood

	BM	PBSC	CB
Typical time frame from initiation of search to transplantation	3-6 mo	3-6 mo	2-4 wk
Usual volume	500-2000 mL	50-300 mL	25-150 mL
Adverse effects for donor	Risks of wound infection, bleeding, general anesthesia, *etc.*	Risks of bleeding, infection, thrombosis, hypotension, electrolyte disturbance, *etc.*	No
Minimal cell dose for transplant	Total nucleated cell: 2 x 10^8/kg	Total $CD34^+$ cell: 2 x 10^6/kg	Total nucleated cell: 2.5 x 10^7/kg
Red blood cell content	High	Low	Low
Possibility to give additional stem cell dose	Possible	Possible	Impossible
Exposure to dimethyl sulfoxide	No if fresh	No if fresh	Yes
HLA matching requirement	More stringent (7-8 out of 8 matched)	More stringent (7-8 out of 8 matched)	Less stringent (4-6 out of 6 matched)
Speed of neutrophil engraftment	About 3 wk	About 2 wk	About 4 wk
Speed of immune reconstitution	Faster	Faster	Slower
Risk of graft-versus-host disease	Medium	Highest	Lowest
Risk of post-transplant infections	Lower	Lower	Higher
Risk of latent virus transmission	Higher	Higher	Lower
Possibility of CMV transmission	Higher as most donors are CMV seropositive	Higher as most donors are CMV seropositive	Lower as most CB units do not harbor CMV
Risk of relapse for high risk patients	Higher	Lower	Higher

PBSC: Peripheral blood stem cell; HLA: Human leukocyte antigen; BM: Bone marrow; CB: Cord blood; CMV: Cytomegalovirus.

established as a standard therapeutic modality for a variety of malignant and non-malignant diseases. The first successful allogeneic HSCT was done with bone marrow (BM) as the source of hematopoietic stem cells in 1968[1]. In the subsequent 2 decades only bone marrow was used as the source of stem cells for transplantation. In the 1960s, experiments have shown that peripheral blood contains a small number of stem cells[2], which can be enriched by pre-treatment with certain chemotherapeutic drugs and hematopoietic growth factors[3-5]. Therefore mobilized peripheral blood stem cells (PBSC) became another stem cell source for HSCT and PBSC has been increasingly used as it has certain advantages compared with BM. In 1978, cord blood (CB) was found to be a rich source of stem cells[6] and was later successfully used for allogeneic HSCT[7] at a lower cell dose infused compared with BM or PBSC.

Nowadays transplant physicians are faced with 3 viable choices of stem cells for allogeneic HSCT, namely BM, PBSC and CB and clinicians have to face the challenges of selecting the optimal stem cell source. Although all 3 sources of stem cells are capable of reconstituting the hematopoietic system in recipient after transplant, they have many inherent differences in cellular constituents and biological and immunological properties. In this article we shall review the advantages and disadvantages of different sources of stem cells and the available clinical evidence that helps clinicians to make decision.

SELECTION AMONG DIFFERENT STEM CELL SOURCES

Although BM, PBSC and CB all contain hematopoietic stem cells, other constituents present in the harvest products before additional manipulation are quite different. Compared with unmanipulated BM, Granulocyte colony stimulating factor-mobilized PBSC and cord blood contain significantly lower amount of red blood cells (RBC) and plasma. This has certain impact on the choice of stem cell source when there is mismatch in blood group between the donor and the recipient, as harvested donor BM must be processed to deplete RBC or plasma or both before infusion to recipient. However, depletion of RBC or plasma is not required for PBSC or cord blood transplants even when blood group is mismatched, as the relatively low amount of RBC and RBC antibodies present in these products are unlikely to cause significant hemolysis. Another important difference among the sources of stem cell is the amount of mature T cells present. PBSC usually contains a lot more mature T cells compared to BM, which in turn contains more T cells compared to CB, and this partly explains the differences in the risk of graft rejection and graft-versus-host disease (GVHD). Depletion of T cells is associated with increased risk of graft rejection and disease relapse, but lower risk of GVHD. The comparison of the characteristics of the 3 different sources of stem cells is presented in Table 1.

We often have to consider and weigh the relative benefits and risks before decision on the source of stem cells for allogeneic HSCT. The selection of stem cell source is often intertwined with the selection of donor. A suggested algorism for selection of donor and stem cell source is given in Figure 1. One of the basic considerations for allogeneic HSCT is whether a Human Leukocyte Antigen (HLA)-matched related donor is available. Although currently results of unrelated donor transplants of many transplant centres are similar to that of matched related donor transplants, the latter is still considered the first choice for most allogeneic HSCTs, as the donor is readily available for initial donation and subsequent back-up, and might be associated with a lower risk of GVHD

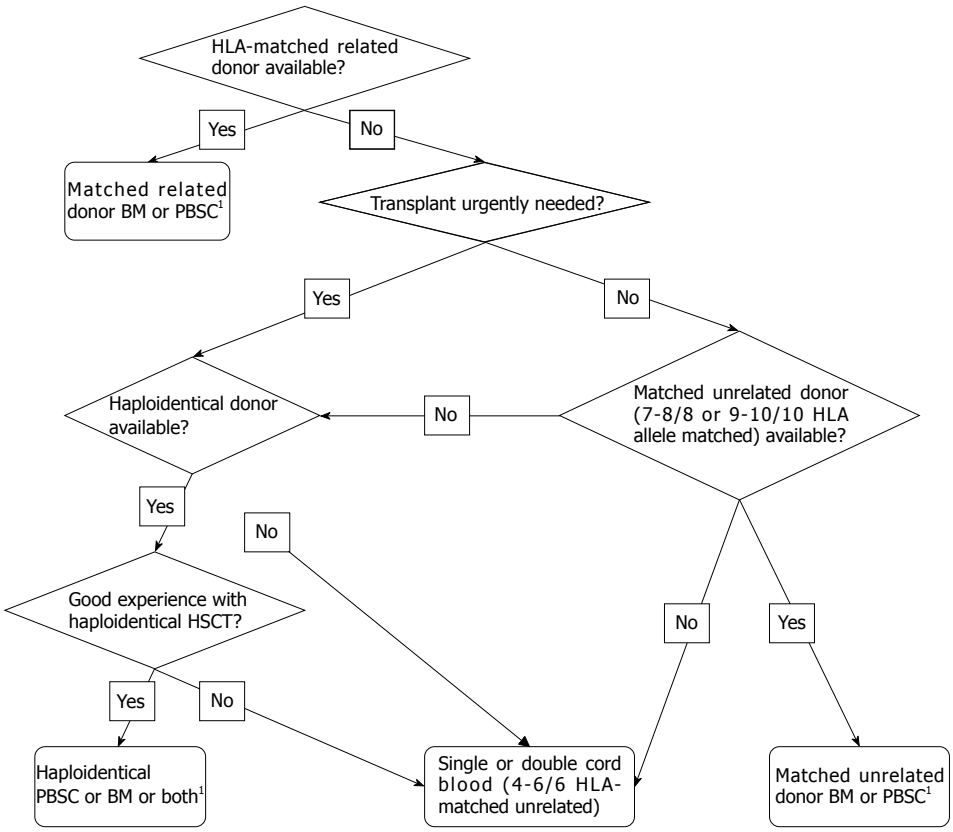

Figure 1 Suggested algorithm for selection of donor and source of stem cells for patients with hematological malignancies. [1]Need to consider pros and cons of BM and PBSC in the context of donor preference, risk of relapse, conditioning regimen, graft-versus-host disease prophylaxis, and graft manipulations, *etc*. PBSC: Peripheral blood stem cell; HSCT: Hematopoietic stem cell transplant; HLA: Human leukocyte antigen; BM: Bone marrow.

and transplant-related mortality (TRM). Therefore, if a matched related donor is available, the choice of stem cell source is simpler and often remains BM versus PBSC, as related donor CB is unlikely to be available. The transplant physician has to weigh the risks and benefits to both the donor and the recipient, explain the different procedures and experiences of stem cell collection to the donor and help the donor to make informed choices. Clinical evidence on different outcomes of recipients transplanted with BM or PBSC presented below will form important basis for the selection.

The donor's perspective should be given due consideration. A prospective study on donors' experience of BM or PBSC donation found that before donation, BM donors had lower confusion, fewer concerns, and were more prepared for donation compared with PBSC donors[8]. Shortly after donation, BM donors experienced more physical side effects than PBSC donors[8]. BM donors also reported greater impact on their social activities, but had better psychological status and were more likely to indicate that the donation made their lives more meaningful[8]. However, there were no significant longer-term differences between BM and PBSC donors including recovery time[8].

In case HLA-matched related donor CB with adequate cell dose is available, relative benefits and risks of CB in comparison to BM or PBSC also need to be considered. If an HLA-matched related donor is not available; we have to find an alternative donor, the choice of which often includes mismatched family donor (including HLA-haploidentical donor), unrelated donor, or unrelated CB. The selection usually depends heavily on the urgency of transplant, HLA matching and cell dose of CB available, and preference and experience of the transplant centre. Unrelated CB and mismatched family donor (BM or PBSC) are usually more readily available compared to unrelated donor and therefore if transplant needs to be done urgently, CB or mismatched family donor is sometimes preferable. If HSCT is not urgently required, unrelated donor BM or PBSC should be given due consideration. Since the requirement for HLA matching is less stringent for unrelated CB compared to BM or PBSC, unrelated CB is preferable to unrelated donor BM or PBSC if no 7-8/8 allele-matched unrelated donor (or 9-10/10 allele-matched unrelated donor which may be associated with even lower risks of TRM and GVHD) is available, provided that the CB is at least 4/6 HLA-matched with adequate cell dose. If there is no single CB with sufficient cell dose, use of double CB can be considered. If transplant is not urgently required and both good matched unrelated donor and unrelated CB with adequate cell dose are available, other considerations prevail, including the preference and experience of the transplant centre, the patient's disease status, the speed of engraftment, risks of infections and GVHD, age, gender and location of donor, ABO blood group matching,

Table 2 Randomized controlled trials comparing bone marrow and peripheral blood stem cell for matched related donor transplant

Ref.	No.of patients	Age of patients (yr)	Underlying diseases	Conditioning	Overall survival (BM *vs* PBSC)	Disease-free survival (BM *vs* PBSC)	Relapse (BM *vs* PBSC)	Transplant-related mortality (BM *vs* PBSC)	Acute graft-versus host disease (BM *vs* PBSC)	Chronic graft-versus host disease (BM *vs* PBSC)	Median time of neutrophil engraftment (d) (BM *vs* PBSC)	Median time of platelet engraftment (d) (BM *vs* PBSC)
[23,29]	56	7-59	Acute leukemias, CML, MDS, MM, NHL	Myeloablative[1]	48% *vs* 56%[2] (2000 d)	50 *vs* 60%[2] (2000 d)	NA	NA	23% *vs* 26%[2] (grades 2-4)	61% *vs* 77%[2] (extensive cGVHD)	18 *vs* 15[a]	18 *vs* 12[b]
[9]	39	22-51	Acute leukemias, CML, CLL, MDS, MM, NHL	Myeloablative[1]	63% *vs* 70%[2] (2 yr)	NA	37% *vs* 0%[a] (2 yr)	32% *vs* 35%[2]	58% *vs* 68%[2] (grades 1-4)	40% *vs* 44%[2] (All cGVHD)	23 *vs* 17.5[b]	18 *vs* 11[d]
[25]	61	15-62	Acute leukemias, CML, MDS, PMF	Bu/Cy	73% *vs* 80%[2] (4 yr)	55% *vs* 80%[2] (4 yr)	30% *vs* 3%[2]	10% *vs* 17%[2]	10% *vs* 21%[2] (grades 2-4)	27% *vs* 56%[2] (All cGVHD)	23 *vs* 17[d]	21 *vs* 13[d]
[10,19]	101	Mean 37	Acute leukemias, CML	Myeloablative[1]	65% *vs* 67%[2] (2 yr)	66% *vs* 67%[2] (2 yr)	15% *vs* 6%[2]	21% *vs* 25%[2]	42% *vs* 44%[2] (grades 2-4)	36% *vs* 65%[b] (All cGVHD) 17% *vs* 44%[b] (extensive cGVHD)	21 *vs* 153	21 *vs* 13[d]
[15-18]	329	19-58	Acute leukemias, CML, MDS	Myeloablative[1]	65% *vs* 65%[2] (2 yr) 65% *vs* 58%[2] (3 yr) 57% *vs* 49%[2] (10 yr)	60% *vs* 56%[2] (3 yr) 46% *vs* 42%[2] (10 yr)	24% *vs* 20%[2] (10 yr)	32% *vs* 24%[2]	42% *vs* 44%[a] (grades 2-4)	56% *vs* 74%[b] (All cGVHD) 19% *vs* 36%[b] (extensive cGVHD)	15 *vs* 12[d]	20 *vs* 15[d]
[12,13,20]	172	12-55	Acute leukemias, CML, CLL, MDS, MM, lymphomas	Myeloablative[1]	54% *vs* 66%[2] (2 yr) 52% *vs* 55%[2] (10 yr)	45% *vs* 65%[a] (2 yr) 40% *vs* 50%[a] (10 yr)	25% *vs* 14%[a] (2 yr) 32% *vs* 20%[a] (10 yr)	30% *vs* 21%[2]	57% *vs* 64%[2] (grades 2-4)	52% *vs* 63%[2] (extensive cGVHD)	21 *vs* 16[d]	19 *vs* 13[d]
[11]	227	19-64	AML, CML, MDS	Bu/Cy	60% *vs* 68%[a] (30 mo)	NA	9% *vs* 9%[2]	32% *vs* 21%[2]	44% *vs* 44%[2] (grades 2-4)	69% *vs* 85%[2] (All cGVHD) 30% *vs* 40%[2] (extensive cGVHD)	23 *vs* 19[d]	22 *vs* 16[d]
[30]	110	15-62	Acute leukemias, MDS, MM, lymphomas	Myeloablative[1]	60% *vs* 34%[a] (4 yr)	NA	13% *vs* 18%[2]	28% *vs* 41%[2]	37% *vs* 52%[2] (grades 2-4)	45% *vs* 61%[2] (All cGVHD) 16% *vs* 28%[2] (extensive cGVHD)	20 *vs* 15[d]	38 *vs* 25[d]
[14]	72	18-61	CML	Myeloablative[1]	72% *vs* 81%[2] (3 yr)	65% *vs* 81%[2] (3 yr)	15% *vs* 0%[a] (3 yr)	20% *vs* 19%[2]	49% *vs* 55%[2] (grades 2-4)	50% *vs* 59%[2] (extensive cGVHD)	22 *vs* 17[a]	21 *vs* 142

[1]Different conditioning regimens; [2]Not statistically significant; [a]$P < 0.05$, [b]$P < 0.01$, [d]$P < 0.01$. GVHD: Graft-versus-host disease; PBSC: Peripheral blood stem cell; HLA: Human leukocyte antigen; BM: Bone marrow; CML: Chronic myeloid leukemia; MDS: Myelodysplastic syndrome; MM: Multiple myeloma; NHL: Non-Hodgkin lymphoma; PMF: Primary myelofibrosis; AML: Acute myeloid leukemia; CLL: Chronic lymphocytic leukemia; Bu/Cy: Busulfan and cyclophosphamide; NA: Not available.

and cytomegalovirus (CMV) status, *etc*. If the recipient is CMV seronegative, CB transplant might be preferred as it is less likely to transmit CMV infection and CMV seronegative donor might not be easily available. Good clinical evidence guiding selection of stem cells for HSCT in patients with hematological malignancies is summarized in the following section.

CLINICAL EVIDENCE FOR SELECTION OF STEM CELL SOURCE IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

HLA-matched related donor

There were a number of randomized controlled trials (RCTs) comparing PBSC and BM as stem cell source in transplants using HLA-matched related donor for patients with hematological malignancies. They are summarized in Table 2. There were no clinical trials comparing HLA-matched related CB with either BM or PBSC.

Most of the RCTs comparing matched related donor BM and PBSC transplantation for patients with hematological malignancies found no significant differences between the two stem cell source in important outcomes including overall survival, disease-free survival, transplant-related mortality, relapse, acute GVHD and chronic GVHD. However, all trials showed significantly faster neutrophil engraftment in PBSC transplants, and all but one trial showed significantly faster platelet engraftment in PBSC transplants, which may result in earlier hospital discharge for PBSC recipients[9,10] and lower cost for PBSC transplantation[10]. Lymphocyte recovery was also found to be better in the PBSC group in one trial[9].

There was one trial showing significantly better overall survival at 30 mo in patients who received PBSC compared with BM[11]. Yet another trial showed opposite result, with better overall survival in BM recipients. However, in this trial CD34 selection was done before stem cell infusion in both BM and PBSC products and PBSC recipients happened to receive more $CD34^+$ cells and T cells. Overall survival at 4 years was significantly worse in the PBSC group compared with the BM group, largely due to increased GVHD and TRM in PBPC recipients receiving T-cells greater than 2×10^5/kg. Acute GVHD appeared strongly associated with increased TRM. Higher number of $CD34^+$ cells was associated with less TRM.

Some trials showed significantly higher probability of relapse in BM recipients than in PBSC recipients[9,12-14], which might translate into better disease-free survival in PBSC transplants compared with BM transplants[12,13]. The differences in disease-free survival appeared more pronounced among patients with higher risk malignancies[12]. "High risk" or "late stage" hematological malignancies usually include patients with acute leukemia in second or later remission, CML in blastic transformation, refractory anemia with excess of blasts in transformation, and lymphoma heavily pretreated with chemotherapy or autologous transplants.

Some trials showed PBSC recipients had significantly more grade 2-4 acute GVHD[15-18], chronic GVHD[15-19] and extensive chronic GVHD[15-19] compared with BM recipients, which resulted in significantly more patients who underwent PBPC transplant needed immunosuppressive treatment[18,20], and longer periods of corticosteroid use and hospitalization[19]. There was no difference in performance status, return to work, incidence of bronchiolitis obliterans, hematopoietic function, and secondary malignancies between the two groups in the long term in one trial[18]. In contrast, another trial showed that late mortality due to chronic GVHD was more frequent in PBSC recipients compared with BM recipients[13].

There were 2 more RCTs that included a few patients with severe aplastic anemia in addition to patients with hematological malignancies[21,22]. One small trial of 30 patients found that PBSC transplant resulted in significantly faster hematopoietic reconstitution, fewer days with neutropenic fever, shorter hospital stay and fewer acute GVHD (6.7% *vs* 46.7%)[21]. Another trial of 57 patients found that the PBSC and the BM groups had similar overall survival at 18 mo (64% *vs* 67%), speed to neutrophil and platelet engraftment, and grade 2-4 acute GVHD (54% *vs* 52%)[22]. However, PBSC transplant resulted in significantly more steroid refractory acute GVHD (32% *vs* 0%), chronic GVHD (90% *vs* 47%), extensive chronic GVHD (80% *vs* 22%) and longer requirement for immunosuppressive therapy[22].

A meta-analysis of 5 RCTs[9-12,16,23] showed that PBSC transplant had significantly higher risk of acute GVHD (RR = 1.23, 95%CI: 1.05-1.45) and chronic GVHD (RR = 1.37, 95%CI: 1.08-1.74) compared with BM transplant[24]. A newer meta-analysis of 7 of RCTs[9-12,16,23,25] showed no difference in mortality between PBSC and BM transplants (OR = 0.81, 95%CI: 0.62-1.05)[26]. However, mortality was significantly lower in PBSC recipients compared with BM recipients in studies that included more patients with intermediate or advanced disease (OR = 0.64, 95%CI: 0.45-0.91)[26]. Subgroup analysis revealed no significant association between mortality and $CD34^+$ cell dose[26].

Another meta-analysis of individual data of 1111 patients from 9 RCTs (both published and unpublished) found that there was no significant difference in overall survival between the PBSC and the BM groups but disease-free survival was significantly higher in the PBSC group (OR = 0.80, 95%CI: 0.67-0.97)[27]. Subgroup analyses showed that both overall survival (OR = 0.64, 95%CI: 0.46-0.90) and disease-free survival (OR = 0.63, 95%CI: 0.45-0.87) were significantly better in patients with late stage disease who received PBSC compared with BM[27]. PBSC transplant led to significantly faster neutrophil engraftment (OR = 0.31, 95%CI: 0.25-0.38) and platelet engraftment (OR = 0.52, 95%CI: 0.44-0.61) compared with BM transplant[27]. PBSC transplant was associated with a significant increase in grade 3-4 acute GVHD (OR = 1.39, 95%CI: 1.03-1.88), chronic GVHD (OR = 1.92, 95%CI: 1.47-2.49), and extensive chronic GVHD (OR = 1.89,

95%CI: 1.47-2.42), but a significant decrease in relapse (OR = 0.71, 95%CI: 0.54-0.93) in both late stage disease (OR = 0.59, 95%CI: 0.38-0.93) and early stage disease (OR = 0.69, 95%CI: 0.49-0.98)[27]. Non-relapse mortality was not significantly different between the PBSC and the BM groups[27]. A decision analysis based on meta-analysis results[27] demonstrated the superiority of PBSC over BM in both overall and quality-adjusted life expectancy[28]. However, BM was found to be the more appropriate strategy if the 1-year relapse probability was below 5%[28].

The most recent meta-analysis which included 11 RCTs[9-11,14,18,20-22,25,29,30] found that PBSC and BM transplants had comparable overall survival (HR = 1.06, 95%CI: 0.81-1.39), disease-free survival (HR =1.04, 95%CI: 0.83-1.30), and TRM (HR = 1.08, 95%CI: 0.56-2.10)[31]. PBSC transplant resulted in significantly better neutrophil engraftment (HR = 2.08, 95%CI: 1.80-2.42) and platelet engraftment (HR = 2.77, 95%CI: 1.78-4.30), but significantly more grade 2-4 acute GVHD (HR = 0.75, 95%CI: 0.63-0.90), grade 3-4 acute GVHD (HR = 0.63, 95%CI: 0.47-0.84), chronic GVHD (HR = 0.70, 95%CI: 0.59-0.83), and extensive chronic GVHD (HR = 0.60, 95%CI: 0.39-0.91). PBSC recipients had significantly lower incidence of relapse (HR = 1.91, 95%CI: 1.34-2.74). A significant inverse relationship was observed between acute GVHD and overall survival.

Unrelated donor

There was an RCT comparing PBSC and BM transplants using HLA-matched unrelated donors after myeloablative or reduced intensity conditioning in 551 patients with hematological malignancies. There was no significant difference between the PBSC and the BM groups in 2-year overall survival (51% *vs* 46%), 2-year disease-free survival, relapse, or acute GVHD[32]. However, PBSC transplant resulted in significantly lower risk of graft failure (3% *vs* 9%) and higher risk of chronic GVHD (53% *vs* 41%), especially extensive chronic GVHD (48% *vs* 32%)[32]. However, another recent non-randomized study found that children who received PBSC or BM did not differ significantly in the incidence of acute and chronic GVHD, which might be related to the use of anti-thymocyte globulin as GVHD prophylaxis[33]. The result indicates that more intensive GVHD prophylaxis is required in PBSC transplant and this might abrogate the difference in GVHD risk between PBSC and BM transplants.

There was no RCT comparing unrelated CB with either BM or PBSC but many non-randomized comparative studies were available. In a meta-analysis[34] of 10 non-randomized clinical trials[35-44] comparing unrelated BM and unrelated CB for HSCT in children and adults with malignant and non-malignant hematological diseases, it was found that BM transplant resulted in significantly better overall survival (HR = 1.28, 95%CI: 1.13-1.44) and TRM (RR = 1.28, 95%CI: 1.03-1.58)[34]. However, CB transplant resulted in significantly lower grade 2-4 acute GVHD (RR = 0.73, 95%CI: 0.64-0.82) and chronic GVHD (RR = 0.70, 95%CI: 0.51-0.97) compared with BM transplant[34]. There was no significant difference in the risk of relapse.

There was a large non-randomized study not included in the above meta-analysis comparing unrelated CB with BM and PBSC in 1525 patients with acute leukemia[45]. Leukemia-free survival in CB transplant was comparable with that after 7-8/8 allele-matched BM or PBSC transplant[45]. However, TRM was significantly higher after CB transplant than after 8/8 allele-matched BM transplant (HR = 1.69, 95%CI: 1.19-2.39) or PBPC transplant (HR = 1.62, 95%CI: 1.18-2.23)[45]. Grade 2-4 acute and chronic GVHD were significantly lower in CB recipients compared with 7-8/8 allele-matched PBPC recipients (HR = 0.57, 95%CI: 0.42-0.77 and HR = 0.38, 95%CI: 0.27-0.53, respectively)[45]. Chronic but not acute GVHD was significantly lower after CB transplant than after 8/8 allele-matched BM transplant (HR = 0.63, 95%CI: 0.44-0.90)[45]. There was no difference among the stem cell sources in the rate of relapse[45].

One comparative study performed disease-specific analysis of the difference between CB transplant and BM transplant in 484 patients with AML and 336 patients with ALL after myeloablative conditioning[44]. Among AML patients, CB recipients had significantly lower overall survival (HR 1.5, 95%CI: 1.0-2.0) and leukemia-free survival (HR = 1.5, 95%CI: 1.1-2.0) compared with BM recipients[44]. TRM and relapse did not differ significantly[44]. Among ALL patients, there was no significant difference between the groups in overall survival, leukemia-free survival, TRM, and relapse[44].

Another study compared unrelated CB transplants with unrelated donor BM or PBSC transplants in adults with ALL in first or second complete remission[46]. This study found no significant differences in the 3-year overall survival between CB (44%), matched (44%) and mismatched (43%) unrelated donor transplants. CB transplants had significantly slower engraftment and less grade 2-4 acute but similar chronic GVHD, disease-free survival, TRM, and relapse[46].

OTHER IMPORTANT CONSIDERATIONS

Double cord blood

In case a single CB unit has insufficient cell dose, 2 CB units can be used, but both are preferably at least 4/6 HLA-matched with the recipient and with each other, and together provide sufficient cell dose. Non-randomized studies comparing double CB transplant with single CB transplant in patients with hematological malignancies usually found that double CB transplant was associated with higher incidence of grade 2 acute GVHD[47-52] and lower incidence of leukemia relapse[48-51,53-55], but there was no significant difference in overall survival, disease-free survival, chronic GVHD and engraftment times[50,52,53,56-59]. However, recently one study found superior overall survival and disease-free survival in addition to lower relapse in patients who received double CB compared with single CB transplant, although TRM

and chronic GVHD were not significantly different[60]. Double CB transplant was also found to be more cost-effective in terms of quality adjusted life years in adults with acute leukemia in first remission in France[60]. On the other hand, intrabone injection of single CB might be associated with faster engraftment (median 23 *vs* 28 d) and lower cumulative incidence of relapse (25% *vs* 29%) compared with intravenous double CB transplant[61].

There were some non-randomized studies comparing double CB transplant with BM or PBSC transplant from other donors. One study on 536 patients with hematological malignancies transplanted with myeloablative conditioning found that 5-year leukemia-free survival was similar in double CB transplant (51%) and other types of donors (either BM or PBSC), including matched related donor (33%), matched unrelated donor (48%), and mismatched unrelated donor (38%)[62]. Non-relapse mortality was highest for double CB (34%), compared with matched related donor (24%), matched unrelated donor (14%), or mismatched unrelated donor (27%)[62]. However, the risk of relapse was lowest in recipients of double CB (15%), compared with matched related donor (43%), matched unrelated donor (37%), or mismatched unrelated donor (35%)[62]. The risks of grade 2-4 acute GVHD and chronic GVHD were also the lowest for double CB (60% and 26%), compared with matched related donor (65% and 47%), matched unrelated donor (80% and 43%), or mismatched unrelated donor (85% and 48%)[62].

Another study on 367 patients with hematological malignancies after myeloablative or non-myeloablative conditioning found that 2-year overall survival, progression-free survival, TRM and grade 2-4 acute GVHD were not significantly different in double CB transplant (65%, 55%, 25% and 43%) as compared to related donor transplant (70%, 66%, 15%, and 27%) and unrelated donor transplant (62%, 55%, 27%, and 39%)[63]. However, late acute or chronic GVHD was significantly lower in double CB transplant (28%) as compared to related donor transplant (31%) and unrelated donor transplant (44%)[63].

A third study compared double CB transplant with 9/10 mismatched unrelated donor BM or PBSC transplants with reduced intensity conditioning for patients with hematological malignancies and found that double CB transplant was associated with lower incidence of extensive chronic GVHD at 2 years compared with unrelated donor transplant (6.4% *vs* 21.4%)[64]. However, both groups were comparable for 2-year overall survival (47.9% *vs* 52.3%), progression-free survival (43.3% *vs* 38.3%), TRM (26% *vs* 24.2%), relapse (34.3% *vs* 37.6%), grade 3-4 acute GVHD (19.1% *vs* 21.4%), and neutrophil engraftment time (median 17 *vs* 16 d)[64].

There were 3 studies comparing double CB transplant with unrelated donor PBSC transplants after reduced intensity conditioning for adult patients with hematological malignancies. The study by Le Bourgeois found that the 2 groups had similar 2-year overall survival (61% *vs* 62%), disease-free survival (50.5% *vs* 59.0%), relapse incidence (23.0% *vs* 35.5%), cumulative incidences of engraftment, grade 2-4 acute and chronic GVHD[65]. However, double CB recipients had significantly higher median time to platelet recovery (38 *vs* 0 d), early mortality before day +100 (20.5% *vs* 4.0%), and 2-year TRM (26.5% *vs* 6.0%) compared with PBSC recipients[65]. The presence of a lymphoid disorder was associated with a significantly higher overall survival[65]. The study by Chen found that the 3-year overall survival and progression-free survival were comparable between double CB and PBSC transplant (46% *vs* 50% and 30% *vs* 40%, respectively), but the cumulative incidence of TRM was significantly higher in double CB transplant (26.9% *vs* 10.4%)[66]. The cumulative incidence of grade 2-4 acute GVHD was not significantly different but the 2-year cumulative incidence of chronic GVHD was significantly lower in double CB transplant compared with PBSC transplant (21.9% *vs* 53.9%)[66]. The study by Jacobson found that there was no significant difference between double CB transplant and PBSC transplant in 2-year overall survival (66% *vs* 68%), progression-free survival (49% *vs* 57%), TRM (11% *vs* 11%), relapse (40% *vs* 32%) and grade 2-4 acute GVHD (21% *vs* 12%)[67]. Double CB recipients had significantly more infections (69% *vs* 33%), both viral (29% *vs* 1%) and bacterial (50% *vs* 8%) infections, but significantly less chronic GVHD (24% *vs* 54%)[67]. Reconstitution of T cells was significantly delayed in double CB recipients compared with PBSC recipients for 1-6 mo post-transplant, including naive and memory $CD4^+$ T cells, regulatory T cells, and $CD8^+$ T cells[67]. In contrast, B cells recovered more rapidly in double CB recipients and B cell number remained significantly greater at 3-24 mo post-transplant[67]. Natural killer (NK) cells also recovered more rapidly in double CB recipients and remained significantly greater at 1-24 mo post-transplant[67].

Haploidentical donor

HLA-haploidentical related donor is an important alternative if no matched related donor is available[68]. Either PBSC or BM can be the stem cell source for haploidentical transplant. Positive selection of $CD34^+$ stem cells from harvested PBSC and infusion of high doses of stem cells successfully overcame HLA barrier with good engraftment rate and low incidence of GVHD[69-78]. Leukemia-free survivals and relapses were better in transplants performed in larger centers[79], and in transplants with natural killer cell killer immunoglobulin like receptor (KIR) mismatch[80]. However, infection risk was high as immunoreconstitution was slow with purified $CD34^+$ cells. Subsequently, negative stem cell selection with depletion of $CD3^+$ T cells with or without depletion of $CD19^+$ B cells achieved similar success of engraftment without excessive GVHD, with myeloablative or reduced intensity conditioning[81-85]. Immune recovery with this method was notably faster with reduced infections[81,85,86]. Unmanipulated T cell replete PBSC and/or BM products could also achieve reasonably good results with intensive GVHD prophylaxis or post-transplant cyclophospha-

mide, despite presence of large amount of T cells[87-96]. A non-randomized comparative study of T cell depleted with T cell replete haploidentical transplants for adult patients with hematological malignancies found that T cell replete transplant resulted in significantly better 1-year overall survival (64% *vs* 30%), progression-free survival (50% *vs* 21%), lower TRM (16% *vs* 42%), chronic GVHD (7% *vs* 18%), and infections, with better reconstitution of T cell subsets[97].

Evolving modifications might further improve outcomes of haploidentical HSCT, such as post-transplant CD8-depleted donor lymphocyte infusion, which could promote immune reconstitution[98]. Post-transplant infusion of regulatory T cells could also promote lymphoid reconstitution with improved immunity to opportunistic pathogens, while preventing GVHD in the absence of any post-transplant immunosuppression, and preserving the graft-versus-leukemia effect[99,100]. Coinfusion of mesenchymal stromal cells could facilitate engraftment without increasing leukemia recurrence after haploidentical HSCT[101,102]. Combining PBSC and BM might also improve engraftment, and reduce TRM[103] and relapse[104]. Suicide-gene-engineered donor lymphocytes might accelerate immune reconstitution while limiting GVHD[105-107]. Selective photodepletion of alloreactive T cells could also enhance immunoreconstitution while preventing GVHD[108]. *Ex vivo* induction of anergy to recipient alloantigen by costimulation blockade was another strategy to limit GVHD[109]. Depletion of T cell receptor alpha-beta positive T cells while retaining gammadelta T cells may reduce GVHD while preserving anti-infective and anti-tumor effects[110]. A two-step approach in which the lymphoid and myeloid portions of the graft are given in two separate steps to control and optimize T cell dosing may further improve results with robust immunoreconstitution, low GVHD and better disease control[111,112].

There are some non-randomized studies comparing haploidentical PBSC or BM transplants with other types of donor or stem cell source. The Blood and Marrow Transplant Clinical Trials Network conducted 2 multicentre trials for patients with leukemia or lymphoma undergoing reduced intensity conditioning allogeneic transplants and found that haploidentical transplant and double CB transplant had comparable 1-year overall survival (62% *vs* 54%), 1-year progression-free survival (48% *vs* 46%), neutrophil engraftment (96% *vs* 94%), and grade 2-4 acute GVHD (32% *vs* 40%)[113]. One-year cumulative TRM was lower in haploidentical transplant compared with double CB transplant (7% *vs* 24%), but relapse rate was higher (45% *vs* 31%)[113].

CONCLUSION

In conclusion, existing high level evidence suggest that there is no significant advantage of PBSC over BM in HLA-matched sibling transplant for patients with hematological malignancies. PBSC transplant probably results in lower risk of relapse and hence better disease-free survival, especially in patients with high risk or late stage disease at the expense of higher risks of both severe acute and chronic GVHD. Existing data are insufficient or inconclusive for firm conclusions in specific subgroups such as a particular disease entity, conditioning regimen or in children. High level evidence is scarce in the unrelated donor setting. The only RCT available suggests that PBSC and BM result in comparable overall and disease-free survivals in patients with hematological malignancies; and PBSC transplant results in lower risk of graft failure but higher risk of chronic GVHD. High level evidence is lacking for CB in comparison to BM or PBSC. The risks and benefits of different sources of stem cells likely change with different conditioning regimen, strategies for prophylaxis and treatment of GVHD and manipulation of grafts. The recent success and rapid advance of double CB transplant and haploidentical BM and PBSC transplants further complicate the selection of optimal stem cell source. Novel therapies for treatment and prophylaxis of GVHD also minimize the key differences between stem cell sources. Advances in graft manipulation and cellular therapies might change the whole paradigm making stem cell source selection less critical, *e.g.*, stem cell enrichment could facilitate engraftment, specific and highly selective depletion of certain lymphocyte subsets and alloreactive cells could minimize GVHD, infusion of mesenchymal stem cells could facilitate engraftment and reduce GVHD, titrated T cell dosing and NK cell therapy might reduce relapse. Detailed counseling of patient and donor regarding risks and benefits in the specific context of the patient and transplant method is of paramount importance for informed decision making.

REFERENCES

1 **Gatti RA**, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet* 1968; **2**: 1366-1369 [PMID: 4177932 DOI: 10.1016/S0140-6736(68)92673-1]

2 **Körbling M**, Freireich EJ. Twenty-five years of peripheral blood stem cell transplantation. *Blood* 2011; **117**: 6411-6416 [PMID: 21460243 DOI: 10.1182/blood-2010-12-322214]

3 **Richman CM**, Weiner RS, Yankee RA. Increase in circulating stem cells following chemotherapy in man. *Blood* 1976; **47**: 1031-1039 [PMID: 1276467]

4 **Socinski MA**, Cannistra SA, Elias A, Antman KH, Schnipper L, Griffin JD. Granulocyte-macrophage colony stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. *Lancet* 1988; **1**: 1194-1198 [PMID: 2897009 DOI: 10.1016/S0140-6736(88)92012-0]

5 **Dührsen U**, Villeval JL, Boyd J, Kannourakis G, Morstyn G, Metcalf D. Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood* 1988; **72**: 2074-2081 [PMID: 3264199]

6 **Prindull G**, Prindull B, Meulen N. Haematopoietic stem cells (CFUc) in human cord blood. *Acta Paediatr Scand* 1978; **67**: 413-416 [PMID: 676726 DOI: 10.1111/j.1651-2227.1978.tb16347.x]

7 **Gluckman E**, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 1989; **321**: 1174-1178 [PMID: 2571931 DOI: 10.1056/NEJM198910263211707]

8 **Switzer GE**, Bruce JG, Harrington D, Haagenson M, Drex-

ler R, Foley A, Confer D, Bishop M, Anderlini P, Rowley S, Leitman SF, Anasetti C, Wingard JR. Health-related quality of life of bone marrow versus peripheral bood stem cell donors: a prespecified subgroup analysis from a phase III RCT-BMTCTN protocol 0201. *Biol Blood Marrow Transplant* 2013 Nov 1; Epub ahead of print [PMID: 24184336 DOI: 10.1016/j.bbmt.2013.10.024]

9 **Powles R**, Mehta J, Kulkarni S, Treleaven J, Millar B, Marsden J, Shepherd V, Rowland A, Sirohi B, Tait D, Horton C, Long S, Singhal S. Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. *Lancet* 2000; **355**: 1231-1237 [PMID: 10770306 DOI: 10.1016/S0140-6736(00)02090-0]

10 **Blaise D**, Kuentz M, Fortanier C, Bourhis JH, Milpied N, Sutton L, Jouet JP, Attal M, Bordigoni P, Cahn JY, Boiron JM, Schuller MP, Moatti JP, Michallet M. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Société Française de Greffe de Moelle. *J Clin Oncol* 2000; **18**: 537-546 [PMID: 10653869]

11 **Couban S**, Simpson DR, Barnett MJ, Bredeson C, Hubesch L, Howson-Jan K, Shore TB, Walker IR, Browett P, Messner HA, Panzarella T, Lipton JH. A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. *Blood* 2002; **100**: 1525-1531 [PMID: 12176866 DOI: 10.1182/blood-2002-01-0048]

12 **Bensinger WI**, Martin PJ, Storer B, Clift R, Forman SJ, Negrin R, Kashyap A, Flowers ME, Lilleby K, Chauncey TR, Storb R, Appelbaum FR. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med* 2001; **344**: 175-181 [PMID: 11172139 DOI: 10.1056/NEJM200101183440303]

13 **Mielcarek M**, Storer B, Martin PJ, Forman SJ, Negrin RS, Flowers ME, Inamoto Y, Chauncey TR, Storb R, Appelbaum FR, Bensinger WI. Long-term outcomes after transplantation of HLA-identical related G-CSF-mobilized peripheral blood mononuclear cells versus bone marrow. *Blood* 2012; **119**: 2675-2678 [PMID: 22308289 DOI: 10.1182/blood-2011-12-396275]

14 **Oehler VG**, Radich JP, Storer B, Blume KG, Chauncey T, Clift R, Snyder DS, Forman SJ, Flowers ME, Martin P, Guthrie KA, Negrin RS, Appelbaum FR, Bensinger W. Randomized trial of allogeneic related bone marrow transplantation versus peripheral blood stem cell transplantation for chronic myeloid leukemia. *Biol Blood Marrow Transplant* 2005; **11**: 85-92 [PMID: 15682068 DOI: 10.1016/j.bbmt.2004.09.010]

15 **Schmitz N**, Bacigalupo A, Hasenclever D, Nagler A, Gluckman E, Clark P, Bourquelot P, Greinix H, Frickhofen N, Ringdén O, Zander A, Apperley JF, Gorin C, Borkett K, Schwab G, Goebel M, Russell NH, Gratwohl A. Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: first results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 1998; **21**: 995-1003 [PMID: 9632272]

16 **Schmitz N**, Beksac M, Hasenclever D, Bacigalupo A, Ruutu T, Nagler A, Gluckman E, Russell N, Apperley JF, Gorin NC, Szer J, Bradstock K, Buzyn A, Clark P, Borkett K, Gratwohl A. Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. *Blood* 2002; **100**: 761-767 [PMID: 12130483 DOI: 10.1182/blood-2001-12-0304]

17 **Schmitz N**, Beksac M, Bacigalupo A, Ruutu T, Nagler A, Gluckman E, Russell N, Apperley J, Szerm J, Bradstock K, Buzyn A, Schlegelberger B, Matcham J, Gratwohl A. Filgrastim-mobilized peripheral blood progenitor cells versus bone marrow transplantation for treating leukemia: 3-year results from the EBMT randomized trial. *Haematologica* 2005; **90**: 643-648 [PMID: 15921379]

18 **Friedrichs B**, Tichelli A, Bacigalupo A, Russell NH, Ruutu T, Shapira MY, Beksac M, Hasenclever D, Socié G, Schmitz N. Long-term outcome and late effects in patients transplanted with mobilised blood or bone marrow: a randomised trial. *Lancet Oncol* 2010; **11**: 331-338 [PMID: 20117965 DOI: 10.1016/S1470-2045(09)70352-3]

19 **Mohty M**, Kuentz M, Michallet M, Bourhis JH, Milpied N, Sutton L, Jouet JP, Attal M, Bordigoni P, Cahn JY, Boiron JM, Blaise D. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. *Blood* 2002; **100**: 3128-3134 [PMID: 12384409 DOI: 10.1182/blood.V100.9.3128]

20 **Flowers ME**, Parker PM, Johnston LJ, Matos AV, Storer B, Bensinger WI, Storb R, Appelbaum FR, Forman SJ, Blume KG, Martin PJ. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. *Blood* 2002; **100**: 415-419 [PMID: 12091330 DOI: 10.1182/blood-2002-01-0011]

21 **Mahmoud H**, Fahmy O, Kamel A, Kamel M, El-Haddad A, El-Kadi D. Peripheral blood vs bone marrow as a source for allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 1999; **24**: 355-358 [PMID: 10467322 DOI: 10.1038/sj.bmt.1701906]

22 **Morton J**, Hutchins C, Durrant S. Granulocyte-colony-stimulating factor (G-CSF)-primed allogeneic bone marrow: significantly less graft-versus-host disease and comparable engraftment to G-CSF-mobilized peripheral blood stem cells. *Blood* 2001; **98**: 3186-3191 [PMID: 11719353]

23 **Vigorito AC**, Azevedo WM, Marques JF, Azevedo AM, Eid KA, Aranha FJ, Lorand-Metze I, Oliveira GB, Correa ME, Reis AR, Miranda EC, de Souza CA. A randomised, prospective comparison of allogeneic bone marrow and peripheral blood progenitor cell transplantation in the treatment of haematological malignancies. *Bone Marrow Transplant* 1998; **22**: 1145-1151 [PMID: 9894716 DOI: 10.1038/sj.bmt.1701510]

24 **Cutler C**, Giri S, Jeyapalan S, Paniagua D, Viswanathan A, Antin JH. Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J Clin Oncol* 2001; **19**: 3685-3691 [PMID: 11504750]

25 **Heldal D**, Tjønnfjord G, Brinch L, Albrechtsen D, Egeland T, Steen R, Solheim BG, Evensen SA. A randomised study of allogeneic transplantation with stem cells from blood or bone marrow. *Bone Marrow Transplant* 2000; **25**: 1129-1136 [PMID: 10849524 DOI: 10.1038/sj.bmt.1702422]

26 **Horan JT**, Liesveld JL, Fernandez ID, Lyman GH, Phillips GL, Lerner NB, Fisher SG. Survival after HLA-identical allogeneic peripheral blood stem cell and bone marrow transplantation for hematologic malignancies: meta-analysis of randomized controlled trials. *Bone Marrow Transplant* 2003; **32**: 293-298 [PMID: 12858201 DOI: 10.1038/sj.bmt.1704112]

27 **Stem Cell Trialists' Collaborative Group**. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. *J Clin Oncol* 2005; **23**: 5074-5087 [PMID: 16051954 DOI: 10.1200/JCO.2005.09.020]

28 **Pidala J**, Anasetti C, Kharfan-Dabaja MA, Cutler C, Sheldon A, Djulbegovic B. Decision analysis of peripheral blood versus bone marrow hematopoietic stem cells for allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2009; **15**: 1415-1421 [PMID: 19822301 DOI: 10.1016/j.bbmt.2009.07.009]

29 **Vigorito AC**, Marques Júnior JF, Aranha FJ, Oliveira GB, Miranda EC, De Souza CA. A randomized, prospective comparison of allogeneic bone marrow and peripheral

blood progenitor cell transplantation in the treatment of hematologic malignancies: an update. *Haematologica* 2001; **86**: 665-666 [PMID: 11418381]

30 **Cornelissen JJ**, van der Holt B, Petersen EJ, Vindelov L, Russel CA, Höglund M, Maertens J, Schouten HC, Braakman E, Steijaert MM, Zijlmans MJ, Slaper-Cortenbach I, Boogaerts MA, Löwenberg B, Verdonck LF. A randomized multicenter comparison of CD34(+)-selected progenitor cells from blood vs from bone marrow in recipients of HLA-identical allogeneic transplants for hematological malignancies. *Exp Hematol* 2003; **31**: 855-864 [PMID: 14550800 DOI: 10.1016/S0301-472X(03)00195-4]

31 **Chang YJ**, Weng CL, Sun LX, Zhao YT. Allogeneic bone marrow transplantation compared to peripheral blood stem cell transplantation for the treatment of hematologic malignancies: a meta-analysis based on time-to-event data from randomized controlled trials. *Ann Hematol* 2012; **91**: 427-437 [PMID: 21789620 DOI: 10.1007/s00277-011-1299-8]

32 **Anasetti C**, Logan BR, Lee SJ, Waller EK, Weisdorf DJ, Wingard JR, Cutler CS, Westervelt P, Woolfrey A, Couban S, Ehninger G, Johnston L, Maziarz RT, Pulsipher MA, Porter DL, Mineishi S, McCarty JM, Khan SP, Anderlini P, Bensinger WI, Leitman SF, Rowley SD, Bredeson C, Carter SL, Horowitz MM, Confer DL. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med* 2012; **367**: 1487-1496 [PMID: 23075175 DOI: 10.1056/NEJMoa1203517]

33 **Meisel R**, Klingebiel T, Dilloo D. Peripheral blood stem cells versus bone marrow in pediatric unrelated donor stem cell transplantation. *Blood* 2013; **121**: 863-865 [PMID: 23372155 DOI: 10.1182/blood-2012-12-469668]

34 **Wang J**, Zhan P, Ouyang J, Chen B, Zhou R, Yang Y. Unrelated donor umbilical cord blood transplantation versus unrelated donor bone marrow transplantation in adult and pediatric patients: A meta-analysis. *Leuk Res* 2010; **34**: 1018-1022 [PMID: 20031212 DOI: 10.1016/j.leukres.2009.11.020]

35 **Rocha V**, Cornish J, Sievers EL, Filipovich A, Locatelli F, Peters C, Remberger M, Michel G, Arcese W, Dallorso S, Tiedemann K, Busca A, Chan KW, Kato S, Ortega J, Vowels M, Zander A, Souillet G, Oakill A, Woolfrey A, Pay AL, Green A, Garnier F, Ionescu I, Wernet P, Sirchia G, Rubinstein P, Chevret S, Gluckman E. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood* 2001; **97**: 2962-2971 [PMID: 11342418]

36 **Barker JN**, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. *Blood* 2001; **97**: 2957-2961 [PMID: 11342417]

37 **Dalle JH**, Duval M, Moghrabi A, Wagner E, Vachon MF, Barrette S, Bernstein M, Champagne J, David M, Demers J, Rousseau P, Winikoff R, Champagne MA. Results of an unrelated transplant search strategy using partially HLA-mismatched cord blood as an immediate alternative to HLA-matched bone marrow. *Bone Marrow Transplant* 2004; **33**: 605-611 [PMID: 14743192 DOI: 10.1038/sj.bmt.1704433]

38 **Jacobsohn DA**, Hewlett B, Ranalli M, Seshadri R, Duerst R, Kletzel M. Outcomes of unrelated cord blood transplants and allogeneic-related hematopoietic stem cell transplants in children with high-risk acute lymphocytic leukemia. *Bone Marrow Transplant* 2004; **34**: 901-907 [PMID: 15361908 DOI: 10.1038/sj.bmt.1704681]

39 **Laughlin MJ**, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, Stevens C, Barker JN, Gale RP, Lazarus HM, Marks DI, van Rood JJ, Scaradavou A, Horowitz MM. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004; **351**: 2265-2275 [PMID: 15564543 DOI: 10.1056/NEJMoa041276]

40 **Rocha V**, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A, Jacobsen N, Ruutu T, de Lima M, Finke J, Frassoni F, Gluckman E. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; **351**: 2276-2285 [PMID: 15564544 DOI: 10.1056/NEJMoa041469]

41 **Takahashi S**, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y, Yamada T, Uchimaru K, Tojo A, Shirafuji N, Kodo H, Tani K, Takahashi T, Yamaguchi T, Asano S. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood* 2004; **104**: 3813-3820 [PMID: 15280199 DOI: 10.1182/blood-2004-03-1001]

42 **Barker JN**, Hough RE, van Burik JA, DeFor TE, MacMillan ML, O'Brien MR, Wagner JE. Serious infections after unrelated donor transplantation in 136 children: impact of stem cell source. *Biol Blood Marrow Transplant* 2005; **11**: 362-370 [PMID: 15846290 DOI: 10.1016/j.bbmt.2005.02.004]

43 **Eapen M**, Rubinstein P, Zhang MJ, Stevens C, Kurtzberg J, Scaradavou A, Loberiza FR, Champlin RE, Klein JP, Horowitz MM, Wagner JE. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet* 2007; **369**: 1947-1954 [PMID: 17560447 DOI: 10.1016/S0140-6736(07)60915-5]

44 **Atsuta Y**, Suzuki R, Nagamura-Inoue T, Taniguchi S, Takahashi S, Kai S, Sakamaki H, Kouzai Y, Kasai M, Fukuda T, Azuma H, Takanashi M, Okamoto S, Tsuchida M, Kawa K, Morishima Y, Kodera Y, Kato S. Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood* 2009; **113**: 1631-1638 [PMID: 19104080 DOI: 10.1182/blood-2008-03-147041]

45 **Eapen M**, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W, Sirvent A, Champlin RE, Chao N, Gee AP, Isola L, Laughlin MJ, Marks DI, Nabhan S, Ruggeri A, Soiffer R, Horowitz MM, Gluckman E, Wagner JE. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol* 2010; **11**: 653-660 [PMID: 20558104 DOI: 10.1016/S1470-2045(10)70127-3]

46 **Marks DI**, Woo KA, Zhong X, Appelbaum FR, Bachanova V, Barker JN, Brunstein CG, Gibson J, Kebriaei P, Lazarus HM, Olsson R, Perales MA, Pidala J, Savani B, Rocha V, Eapen M. Unrelated umbilical cord blood transplant for adult acute lymphoblastic leukemia in first and second complete remission: a comparison with allografts from adult unrelated donors. *Haematologica* 2013 Sep 20; Epub ahead of print [PMID: 24056817 DOI: 10.3324/haematol.2013.094193]

47 **MacMillan ML**, Weisdorf DJ, Brunstein CG, Cao Q, DeFor TE, Verneris MR, Blazar BR, Wagner JE. Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: analysis of risk factors. *Blood* 2009; **113**: 2410-2415 [PMID: 18997171 DOI: 10.1182/blood-2008-07-163238]

48 **Brunstein CG**, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR, McGlave PB, Wagner JE. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. *Blood* 2007; **110**: 3064-3070 [PMID: 17569820 DOI: 10.1182/blood-2007-04-067215]

49 **Verneris MR**, Brunstein CG, Barker J, MacMillan ML, DeFor T, McKenna DH, Burke MJ, Blazar BR, Miller JS, McGlave PB, Weisdorf DJ, Wagner JE. Relapse risk after umbilical cord blood transplantation: enhanced graft-versus-leukemia effect in recipients of 2 units. *Blood* 2009; **114**: 4293-4299 [PMID: 19706886 DOI: 10.1182/blood-2009-05-220525]

50 **Sideri A**, Neokleous N, Brunet De La Grange P, Guerton B,

Le Bousse Kerdilles MC, Uzan G, Peste-Tsilimidos C, Gluckman E. An overview of the progress on double umbilical cord blood transplantation. *Haematologica* 2011; **96**: 1213-1220 [PMID: 21546497 DOI: 10.3324/haematol.2010.038836]

51 **Sanz J**, Wagner JE, Sanz MA, Defor T, Montesinos P, Bachanova V, Lorenzo I, Warlick E, Sanz GF, Brunstein C. Myeloablative cord blood transplantation in adults with acute leukemia: comparison of two different transplant platforms. *Biol Blood Marrow Transplant* 2013; **19**: 1725-1730 [PMID: 24090598 DOI: 10.1016/j.bbmt.2013.09.015]

52 **Ruggeri A**, Sanz G, Bittencourt H, Sanz J, Rambaldi A, Volt F, Yakoub-Agha I, Ribera JM, Mannone L, Sierra J, Mohty M, Solano C, Nabhan S, Arcese W, Gluckman E, Labopin M, Rocha V. Comparison of outcomes after single or double cord blood transplantation in adults with acute leukemia using different types of myeloablative conditioning regimen, a retrospective study on behalf of Eurocord and the Acute Leukemia Working Party of EBMT. *Leukemia* 2013 Sep 5; Epub ahead of print [PMID: 24005245 DOI: 10.1038/leu.2013.259]

53 **Kindwall-Keller TL**, Hegerfeldt Y, Meyerson HJ, Margevicius S, Fu P, van Heeckeren W, Lazarus HM, Cooper BW, Gerson SL, Barr P, Tse WW, Curtis C, Fanning LR, Creger RJ, Carlson-Barko JM, Laughlin MJ. Prospective study of one- vs two-unit umbilical cord blood transplantation following reduced intensity conditioning in adults with hematological malignancies. *Bone Marrow Transplant* 2012; **47**: 924-933 [PMID: 22002488 DOI: 10.1038/bmt.2011.195]

54 **Rodrigues CA**, Sanz G, Brunstein CG, Sanz J, Wagner JE, Renaud M, de Lima M, Cairo MS, Fürst S, Rio B, Dalley C, Carreras E, Harousseau JL, Mohty M, Taveira D, Dreger P, Sureda A, Gluckman E, Rocha V. Analysis of risk factors for outcomes after unrelated cord blood transplantation in adults with lymphoid malignancies: a study by the Eurocord-Netcord and lymphoma working party of the European group for blood and marrow transplantation. *J Clin Oncol* 2009; **27**: 256-263 [PMID: 19064984 DOI: 10.1200/JCO.2007.15.8865]

55 **Avery S**, Barker JN. Cord blood transplants: one, two or more units? *Curr Opin Hematol* 2010; **17**: 531-537 [PMID: 20739881 DOI: 10.1097/MOH.0b013e32833e5b59]

56 **Scaradavou A**, Brunstein CG, Eapen M, Le-Rademacher J, Barker JN, Chao N, Cutler C, Delaney C, Kan F, Isola L, Karanes C, Laughlin MJ, Wagner JE, Shpall EJ. Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. *Blood* 2013; **121**: 752-758 [PMID: 23223509 DOI: 10.1182/blood-2012-08-449108]

57 **Goldstein G**, Elhasid R, Bielorai B, Shimoni A, Yerushalmi R, Kassis I, Nagler A. Adults requiring cord blood transplants but have insufficient cell doses from a single cord blood unit can receive two units with successful engraftment kinetics similar to those of children receiving a single unit. *Leuk Lymphoma* 2011; **52**: 635-641 [PMID: 21338286 DOI: 10.3109/10428194.2010.548109]

58 **Yoo KH**, Lee SH, Sung KW, Koo HH, Chung NG, Cho B, Kim HK, Kang HJ, Shin HY, Ahn HS, Baek HJ, Han DK, Kook H, Hwang TJ, Kim SY, Lee YH, Hah JO, Im HJ, Seo JJ, Park SK, Jung HJ, Park JE, Lim YJ, Park SS, Lim YT, Yoo ES, Ryu KH, Park HJ, Park BK. Current status of pediatric umbilical cord blood transplantation in Korea: a multicenter retrospective analysis of 236 cases. *Am J Hematol* 2011; **86**: 12-17 [PMID: 21064135 DOI: 10.1002/ajh.21886]

59 **Robin M**, Sanz GF, Ionescu I, Rio B, Sirvent A, Renaud M, Carreras E, Milpied N, Mohty M, Beguin Y, Bordigoni P, de Witte T, Picardi A, Purtill D, Gluckman E, Kroger N, Rocha V. Unrelated cord blood transplantation in adults with myelodysplasia or secondary acute myeloblastic leukemia: a survey on behalf of Eurocord and CLWP of EBMT. *Leukemia* 2011; **25**: 75-81 [PMID: 20882048 DOI: 10.1038/leu.2010.219]

60 **Labopin M**, Ruggeri A, Gorin NC, Gluckman E, Blaise D, Mannone L, Milpied N, Yakoub-Agha I, Deconinck E, Michallet M, Fegueux N, Socié G, Nguyen S, Cahn JY, de Revel T, Garnier F, Faucher C, Tarighт N, Kenzey C, Volt F, Bertrand D, Mohty M, Rocha V. Cost-effectiveness and clinical outcomes of double versus single cord bloodtransplantation in adults with acute leukemia in France. *Haematologica* 2013 Oct 18; Epub ahead of print [PMID: 24143000 DOI: 10.3324/haematol.2013.092254]

61 **Rocha V**, Labopin M, Ruggeri A, Podestà M, Gallamini A, Bonifazi F, Sanchez-Guijo FM, Rovira M, Socie G, Baltadakis I, Michallet M, Deconinck E, Bacigalupo A, Mohty M, Gluckman E, Frassoni F. Unrelated cord blood transplantation: outcomes after single-unit intrabone injection compared with double-unit intravenous injection in patients with hematological malignancies. *Transplantation* 2013; **95**: 1284-1291 [PMID: 23507699 DOI: 10.1097/TP.0b013e318288ca4d]

62 **Brunstein CG**, Gutman JA, Weisdorf DJ, Woolfrey AE, Defor TE, Gooley TA, Verneris MR, Appelbaum FR, Wagner JE, Delaney C. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood* 2010; **116**: 4693-4699 [PMID: 20686119 DOI: 10.1182/blood-2010-05-285304]

63 **Ponce DM**, Zheng J, Gonzales AM, Lubin M, Heller G, Castro-Malaspina H, Giralt S, Hsu K, Jakubowski AA, Jenq RR, Koehne G, Papadopoulos EB, Perales MA, van den Brink MR, Young JW, Boulad F, Kernan NA, Kobos R, Prockop S, Scaradavou A, Small T, O'Reilly RJ, Barker JN. Reduced late mortality risk contributes to similar survival after double-unit cord blood transplantation compared with related and unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2011; **17**: 1316-1326 [PMID: 21232625 DOI: 10.1016/j.bbmt.2011.01.006]

64 **Malard F**, Fürst S, Loirat M, Chevallier P, El-Cheikh J, Guillaume T, Delaunay J, Le Gouill S, Moreau P, Blaise D, Mohty M. Effect of graft source on mismatched unrelated donor hemopoietic stem cell transplantation after reduced intensity conditioning. *Leukemia* 2013; **27**: 2113-2117 [PMID: 23752174 DOI: 10.1038/leu.2013.170]

65 **Le Bourgeois A**, Mohr C, Guillaume T, Delaunay J, Malard F, Loirat M, Peterlin P, Blin N, Dubruille V, Mahe B, Gastinne T, Le Gouill S, Moreau P, Mohty M, Planche L, Lode L, Bene MC, Chevallier P. Comparison of outcomes after two standards-of-care reduced-intensity conditioning regimens and two different graft sources for allogeneic stem cell transplantation in adults with hematologic diseases: a single-center analysis. *Biol Blood Marrow Transplant* 2013; **19**: 934-939 [PMID: 23523970 DOI: 10.1016/j.bbmt.2013.03.009]

66 **Chen YB**, Aldridge J, Kim HT, Ballen KK, Cutler C, Kao G, Liney D, Bourdeau G, Alyea EP, Armand P, Koreth J, Ritz J, Spitzer TR, Soiffer RJ, Antin JH, Ho VT. Reduced-intensity conditioning stem cell transplantation: comparison of double umbilical cord blood and unrelated donor grafts. *Biol Blood Marrow Transplant* 2012; **18**: 805-812 [PMID: 22015993 DOI: 10.1016/j.bbmt.2011.10.016]

67 **Jacobson CA**, Turki AT, McDonough SM, Stevenson KE, Kim HT, Kao G, Herrera MI, Reynolds CG, Alyea EP, Ho VT, Koreth J, Armand P, Chen YB, Ballen K, Soiffer RJ, Antin JH, Cutler CS, Ritz J. Immune reconstitution after double umbilical cord blood stem cell transplantation: comparison with unrelated peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2012; **18**: 565-574 [PMID: 21875503 DOI: 10.1016/j.bbmt.2011.08.018]

68 **Di Bartolomeo P**, Santarone S, De Angelis G, Picardi A, Cudillo L, Cerretti R, Adorno G, Angelini S, Andreani M, De Felice L, Rapanotti MC, Sarmati L, Bavaro P, Papalinetti G, Di Nicola M, Papola F, Montanari M, Nagler A, Arcese W. Haploidentical, unmanipulated, G-CSF-primed bone marrow transplantation for patients with high-risk hematologic malignancies. *Blood* 2013; **121**: 849-857 [PMID: 23165479 DOI:

10.1182/blood-2012-08-453399]
69 **Aversa F**, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S, Felicini R, Falcinelli F, Velardi A, Ruggeri L, Aloisi T, Saab JP, Santucci A, Perruccio K, Martelli MP, Mecucci C, Reisner Y, Martelli MF. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol* 2005; **23**: 3447-3454 [PMID: 15753458 DOI: 10.1200/JCO.2005.09.117]
70 **Ciurea SO**, Saliba R, Rondon G, Pesoa S, Cano P, Fernandez-Vina M, Qureshi S, Worth LL, McMannis J, Kebriaei P, Jones RB, Korbling M, Qazilbash M, Shpall EJ, Giralt S, de Lima M, Champlin RE, Gajewski J. Reduced-intensity conditioning using fludarabine, melphalan and thiotepa for adult patients undergoing haploidentical SCT. *Bone Marrow Transplant* 2010; **45**: 429-436 [PMID: 19668237 DOI: 10.1038/bmt.2009.189]
71 **Aversa F**, Terenzi A, Felicini R, Tabilio A, Falzetti F, Carotti A, Falcinelli F, Sodani P, Amici A, Zucchetti P, Mazzarino I, Martelli MF. Mismatched T cell-depleted hematopoietic stem cell transplantation for children with high-risk acute leukemia. *Bone Marrow Transplant* 1998; **22** Suppl 5: S29-S32 [PMID: 9989886]
72 **Handgretinger R**, Klingebiel T, Lang P, Schumm M, Neu S, Geiselhart A, Bader P, Schlegel PG, Greil J, Stachel D, Herzog RJ, Niethammer D. Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children. *Bone Marrow Transplant* 2001; **27**: 777-783 [PMID: 11477433 DOI: 10.1038/sj.bmt.1702996]
73 **Peters C**, Matthes-Martin S, Fritsch G, Holter W, Lion T, Witt V, Höcker P, Fischer G, Dieckmann K, Handgretinger R, Klingebiel T, Gadner H. Transplantation of highly purified peripheral blood CD34+ cells from HLA-mismatched parental donors in 14 children: evaluation of early monitoring of engraftment. *Leukemia* 1999; **13**: 2070-2078 [PMID: 10602431]
74 **Ortín M**, Raj R, Kinning E, Williams M, Darbyshire PJ. Partially matched related donor peripheral blood progenitor cell transplantation in paediatric patients adding fludarabine and anti-lymphocyte gamma-globulin. *Bone Marrow Transplant* 2002; **30**: 359-366 [PMID: 12235520 DOI: 10.1038/sj.bmt.1703667]
75 **Lang P**, Greil J, Bader P, Handgretinger R, Klingebiel T, Schumm M, Schlegel PG, Feuchtinger T, Pfeiffer M, Scheel-Walter H, Führer M, Martin D, Niethammer D. Long-term outcome after haploidentical stem cell transplantation in children. *Blood Cells Mol Dis* 2004; **33**: 281-287 [PMID: 15528145 DOI: 10.1016/j.bcmd.2004.08.017]
76 **Klingebiel T**, Handgretinger R, Lang P, Bader P, Niethammer D. Haploidentical transplantation for acute lymphoblastic leukemia in childhood. *Blood Rev* 2004; **18**: 181-192 [PMID: 15183902 DOI: 10.1016/S0268-960X(03)00063-8]
77 **Marks DI**, Khattry N, Cummins M, Goulden N, Green A, Harvey J, Hunt LP, Keen L, Robinson SP, Steward CG, Cornish JM. Haploidentical stem cell transplantation for children with acute leukaemia. *Br J Haematol* 2006; **134**: 196-201 [PMID: 16846478 DOI: 10.1111/j.1365-2141.2006.06140.x]
78 **Reisner Y**, Martelli MF. Tolerance induction by 'megadose' transplants of CD34+ stem cells: a new option for leukemia patients without an HLA-matched donor. *Curr Opin Immunol* 2000; **12**: 536-541 [PMID: 11007356]
79 **Klingebiel T**, Cornish J, Labopin M, Locatelli F, Darbyshire P, Handgretinger R, Balduzzi A, Owoc-Lempach J, Fagioli F, Or R, Peters C, Aversa F, Polge E, Dini G, Rocha V. Results and factors influencing outcome after fully haploidentical hematopoietic stem cell transplantation in children with very high-risk acute lymphoblastic leukemia: impact of center size: an analysis on behalf of the Acute Leukemia and Pediatric Disease Working Parties of the European Blood and Marrow Transplant group. *Blood* 2010; **115**: 3437-3446 [PMID: 20040760 DOI: 10.1182/blood-2009-03-207001]
80 **Symons HJ**, Leffell MS, Rossiter ND, Zahurak M, Jones RJ, Fuchs EJ. Improved survival with inhibitory killer immunoglobulin receptor (KIR) gene mismatches and KIR haplotype B donors after nonmyeloablative, HLA-haploidentical bone marrow transplantation. *Biol Blood Marrow Transplant* 2010; **16**: 533-542 [PMID: 19961944 DOI: 10.1016/j.bbmt.2009.11.022]
81 **Lang P**, Schumm M, Greil J, Bader P, Klingebiel T, Müller I, Feuchtinger T, Pfeiffer M, Schlegel PG, Niethammer D, Handgretinger R. A comparison between three graft manipulation methods for haploidentical stem cell transplantation in pediatric patients: preliminary results of a pilot study. *Klin Padiatr* 2005; **217**: 334-338 [PMID: 16307419 DOI: 10.1055/s-2005-872529]
82 **Federmann B**, Bornhauser M, Meisner C, Kordelas L, Beelen DW, Stuhler G, Stelljes M, Schwerdtfeger R, Christopeit M, Behre G, Faul C, Vogel W, Schumm M, Handgretinger R, Kanz L, Bethge WA. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: a phase II study. *Haematologica* 2012; **97**: 1523-1531 [PMID: 22491731 DOI: 10.3324/haematol.2011.059378]
83 **Palma J**, Salas L, Carrión F, Sotomayor C, Catalán P, Paris C, Turner V, Jorquera H, Handgretinger R, Rivera GK. Haploidentical stem cell transplantation for children with high-risk leukemia. *Pediatr Blood Cancer* 2012; **59**: 895-901 [PMID: 22238059 DOI: 10.1002/pbc.24022]
84 **Chen DF**, Prasad VK, Broadwater G, Reinsmoen NL, DeOliveira A, Clark A, Sullivan KM, Chute JP, Horwitz ME, Gasparetto C, Long GD, Yang Y, Chao NJ, Rizzieri DA. Differential impact of inhibitory and activating Killer Ig-Like Receptors (KIR) on high-risk patients with myeloid and lymphoid malignancies undergoing reduced intensity transplantation from haploidentical related donors. *Bone Marrow Transplant* 2012; **47**: 817-823 [PMID: 22139069 DOI: 10.1038/bmt.2011.181]
85 **Handgretinger R**, Chen X, Pfeiffer M, Mueller I, Feuchtinger T, Hale GA, Lang P. Feasibility and outcome of reduced-intensity conditioning in haploidentical transplantation. *Ann N Y Acad Sci* 2007; **1106**: 279-289 [PMID: 17442774 DOI: 10.1196/annals.1392.022]
86 **Chen X**, Hale GA, Barfield R, Benaim E, Leung WH, Knowles J, Horwitz EM, Woodard P, Kasow K, Yusuf U, Behm FG, Hayden RT, Shurtleff SA, Turner V, Srivastava DK, Handgretinger R. Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical stem cell graft for paediatric refractory haematological malignancies. *Br J Haematol* 2006; **135**: 524-532 [PMID: 17010105 DOI: 10.1111/j.1365-2141.2006.06330.x]
87 **Raiola AM**, Dominietto A, Ghiso A, Di Grazia C, Lamparelli T, Gualandi F, Bregante S, Van Lint MT, Geroldi S, Luchetti S, Ballerini F, Miglino M, Varaldo R, Bacigalupo A. Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. *Biol Blood Marrow Transplant* 2013; **19**: 117-122 [PMID: 22940057 DOI: 10.1016/j.bbmt.2012.08.014]
88 **Solomon SR**, Sizemore CA, Sanacore M, Zhang X, Brown S, Holland HK, Morris LE, Bashey A. Haploidentical transplantation using T cell replete peripheral blood stem cells and myeloablative conditioning in patients with high-risk hematologic malignancies who lack conventional donors is well tolerated and produces excellent relapse-free survival: results of a prospective phase II trial. *Biol Blood Marrow Transplant* 2012; **18**: 1859-1866 [PMID: 22863841 DOI: 10.1016/j.bbmt.2012.06.019]
89 **Wang HX**, Yan HM, Wang ZD, Xue M, Liu J, Guo ZK. Haploidentical hematopoietic stem cell transplantation in hematologic malignancies with G-CSF mobilized bone marrow plus peripheral blood stem cells grafts without T cell

depletion: a single center report of 29 cases. *Leuk Lymphoma* 2012; **53**: 654-659 [PMID: 21929286 DOI: 10.3109/10428194.2011.624225]

90 **Munchel AT**, Kasamon YL, Fuchs EJ. Treatment of hematological malignancies with nonmyeloablative, HLA-haploidentical bone marrow transplantation and high dose, post-transplantation cyclophosphamide. *Best Pract Res Clin Haematol* 2011; **24**: 359-368 [PMID: 21925089 DOI: 10.1016/j.beha.2011.05.001]

91 **Chen Y**, Liu K, Xu L, Chen H, Liu D, Zhang X, Shi H, Han W, Wang Y, Zhao T, Wang J, Wang J, Huang X. HLA-mismatched hematopoietic SCT without in vitro T-cell depletion for myelodysplastic syndrome. *Bone Marrow Transplant* 2010; **45**: 1333-1339 [PMID: 20062100 DOI: 10.1038/bmt.2009.351]

92 **Wang HX**, Yan HM, Duan LN, Wang ZD, Zhu L, Xue M, Liu J, Hu LD, Guo ZK. Haploidentical hematopoietic stem cell transplantation in child hematologic malignancies with G-CSF-mobilized marrow grafts without T-cell depletion: a single-center report of 45 cases. *Pediatr Hematol Oncol* 2009; **26**: 119-128 [PMID: 19382033]

93 **Luznik L**, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, Gooley TA, Piantadosi S, Kaup M, Ambinder RF, Huff CA, Matsui W, Bolaños-Meade J, Borrello I, Powell JD, Harrington E, Warnock S, Flowers M, Brodsky RA, Sandmaier BM, Storb RF, Jones RJ, Fuchs EJ. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant* 2008; **14**: 641-650 [PMID: 18489989 DOI: 10.1016/j.bbmt.2008.03.005]

94 **Liu D**, Huang X, Liu K, Xu L, Chen H, Han W, Chen Y, Zhang X, Jiang Q. Haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for treatment of hematological malignancies in children. *Biol Blood Marrow Transplant* 2008; **14**: 469-477 [PMID: 18342790 DOI: 10.1016/j.bbmt.2008.02.007]

95 **Yoshihara T**, Okada K, Kobayashi M, Kikuta A, Kato K, Adachi N, Kikuchi A, Ishida H, Hirota Y, Kuroda H, Nagatoshi Y, Inukai T, Koike K, Kigasawa H, Yagasaki H, Tokuda K, Kishimoto T, Nakano T, Fujita N, Goto H, Nakazawa Y, Kanegane H, Matsuzaki A, Osugi Y, Hasegawa D, Uoshima N, Nakamura K, Tsuchida M, Tanaka R, Watanabe A, Yabe H. Outcome of non-T-cell-depleted HLA-haploidentical hematopoietic stem cell transplantation from family donors in children and adolescents. *Int J Hematol* 2007; **85**: 246-255 [PMID: 17483063 DOI: 10.1532/IJH97.06185]

96 **Bashey A**, Zhang X, Sizemore CA, Manion K, Brown S, Holland HK, Morris LE, Solomon SR. T-cell-replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. *J Clin Oncol* 2013; **31**: 1310-1316 [PMID: 23423745 DOI: 10.1200/JCO.2012.44.3523]

97 **Ciurea SO**, Mulanovich V, Saliba RM, Bayraktar UD, Jiang Y, Bassett R, Wang SA, Konopleva M, Fernandez-Vina M, Montes N, Bosque D, Chen J, Rondon G, Alatrash G, Alousi A, Bashir Q, Korbling M, Qazilbash M, Parmar S, Shpall E, Nieto Y, Hosing C, Kebriaei P, Khouri I, Popat U, de Lima M, Champlin RE. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2012; **18**: 1835-1844 [PMID: 22796535 DOI: 10.1016/j.bbmt.2012.07.003]

98 **Dodero A**, Carniti C, Raganato A, Vendramin A, Farina L, Spina F, Carlo-Stella C, Di Terlizzi S, Milanesi M, Longoni P, Gandola L, Lombardo C, Corradini P. Haploidentical stem cell transplantation after a reduced-intensity conditioning regimen for the treatment of advanced hematologic malignancies: posttransplantation CD8-depleted donor lymphocyte infusions contribute to improve T-cell recovery . *Blood* 2009; **113**: 4771-4779 [PMID: 19211934 DOI: 10.1182/blood-2008-10-183723]

99 **Di Ianni M**, Falzetti F, Carotti A, Terenzi A, Castellino F, Bonifacio E, Del Papa B, Zei T, Ostini RI, Cecchini D, Aloisi T, Perruccio K, Ruggeri L, Balucani C, Pierini A, Sportoletti P, Aristei C, Falini B, Reisner Y, Velardi A, Aversa F, Martelli MF. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* 2011; **117**: 3921-3928 [PMID: 21292771 DOI: 10.1182/blood-2010-10-311894]

100 **Di Ianni M**, Falzetti F, Carotti A, Terenzi A, Del Papa B, Perruccio K, Ruggeri L, Sportoletti P, Rosati E, Marconi P, Falini B, Reisner Y, Velardi A, Aversa F, Martelli MF. Immunoselection and clinical use of T regulatory cells in HLA-haploidentical stem cell transplantation. *Best Pract Res Clin Haematol* 2011; **24**: 459-466 [PMID: 21925099 DOI: 10.1016/j.beha.2011.05.005]

101 **Liu K**, Chen Y, Zeng Y, Xu L, Liu D, Chen H, Zhang X, Han W, Wang Y, Zhao T, Wang J, Wang J, Han Q, Zhao C, Huang X. Coinfusion of mesenchymal stromal cells facilitates platelet recovery without increasing leukemia recurrence in haploidentical hematopoietic stem cell transplantation: a randomized, controlled clinical study. *Stem Cells Dev* 2011; **20**: 1679-1685 [PMID: 21142788 DOI: 10.1089/scd.2010.0447]

102 **Ball LM**, Bernardo ME, Roelofs H, Lankester A, Cometa A, Egeler RM, Locatelli F, Fibbe WE. Cotransplantation of ex vivo expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. *Blood* 2007; **110**: 2764-2767 [PMID: 17638847 DOI: 10.1182/blood-2007-04-087056]

103 **Xu LP**, Liu KY, Liu DH, Chen H, Han W, Chen YH, Wang Y, Huang XJ. The inferiority of G-PB to rhG-CSF-mobilized blood and marrow grafts as a stem cell source in patients with high-risk acute leukemia who underwent unmanipulated HLA-mismatched/haploidentical transplantation: a comparative analysis. *Bone Marrow Transplant* 2010; **45**: 985-992 [PMID: 19898505 DOI: 10.1038/bmt.2009.311]

104 **Yan HM**, Xue M, Wang ZD, Zhu L, Liu J, Ding L, Pan SP, Duan LN, Wang HX. [Haploidentical allogeneic bone marrow stem cell transplantation combined with peripheral blood stem cells for therapy of leukemia]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2009; **17**: 1330-1334 [PMID: 19840477]

105 **Vago L**, Oliveira G, Bondanza A, Noviello M, Soldati C, Ghio D, Brigida I, Greco R, Lupo Stanghellini MT, Peccatori J, Fracchia S, Del Fiacco M, Traversari C, Aiuti A, Del Maschio A, Bordignon C, Ciceri F, Bonini C. T-cell suicide gene therapy prompts thymic renewal in adults after hematopoietic stem cell transplantation. *Blood* 2012; **120**: 1820-1830 [PMID: 22709689 DOI: 10.1182/blood-2012-01-405670]

106 **Ciceri F**, Bonini C, Stanghellini MT, Bondanza A, Traversari C, Salomoni M, Turchetto L, Colombi S, Bernardi M, Peccatori J, Pescarollo A, Servida P, Magnani Z, Perna SK, Valtolina V, Crippa F, Callegaro L, Spoldi E, Crocchiolo R, Fleischhauer K, Ponzoni M, Vago L, Rossini S, Santoro A, Todisco E, Apperley J, Olavarria E, Slavin S, Weissinger EM, Ganser A, Stadler M, Yannaki E, Fassas A, Anagnostopoulos A, Bregni M, Stampino CG, Bruzzi P, Bordignon C. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study. *Lancet Oncol* 2009; **10**: 489-500 [PMID: 19345145 DOI: 10.1016/S1470-2045(09)70074-9]

107 **Di Stasi A**, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, Straathof K, Liu E, Durett AG, Grilley B, Liu H, Cruz CR, Savoldo B, Gee AP, Schindler J, Krance RA, Heslop HE, Spencer DM, Rooney CM, Brenner MK. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl*

J Med 2011; **365**: 1673-1683 [PMID: 22047558 DOI: 10.1056/NEJMoa1106152]

108 **Bastien JP**, Roy J, Roy DC. Selective T-cell depletion for haplotype-mismatched allogeneic stem cell transplantation. *Semin Oncol* 2012; **39**: 674-682 [PMID: 23206844 DOI: 10.1053/j.seminoncol.2012.09.004]

109 **Guinan EC**, Boussiotis VA, Neuberg D, Brennan LL, Hirano N, Nadler LM, Gribben JG. Transplantation of anergic histoincompatible bone marrow allografts. *N Engl J Med* 1999; **340**: 1704-1714 [PMID: 10352162 DOI: 10.1056/NEJM199906033402202]

110 **Handgretinger R**. New approaches to graft engineering for haploidentical bone marrow transplantation. *Semin Oncol* 2012; **39**: 664-673 [PMID: 23206843 DOI: 10.1053/j.seminoncol.2012.09.007]

111 **Grosso D**, Flomenberg N. A two-step approach to allogeneic haploidentical hematopoietic stem cell transplantation. *Semin Oncol* 2012; **39**: 694-706 [PMID: 23206846 DOI: 10.1053/j.seminoncol.2012.09.006]

112 **Grosso D**, Carabasi M, Filicko-O'Hara J, Kasner M, Wagner JL, Colombe B, Cornett Farley P, O'Hara W, Flomenberg P, Werner-Wasik M, Brunner J, Mookerjee B, Hyslop T, Weiss M, Flomenberg N. A 2-step approach to myeloablative haploidentical stem cell transplantation: a phase 1/2 trial performed with optimized T-cell dosing. *Blood* 2011; **118**: 4732-4739 [PMID: 21868572 DOI: 10.1182/blood-2011-07-365338]

113 **Brunstein CG**, Fuchs EJ, Carter SL, Karanes C, Costa LJ, Wu J, Devine SM, Wingard JR, Aljitawi OS, Cutler CS, Jagasia MH, Ballen KK, Eapen M, O'Donnell PV. Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood* 2011; **118**: 282-288 [PMID: 21527516 DOI: 10.1182/blood-2011-03-344853]

P- Reviewers: Goebel WS, Lee ACW, Tommasini A
S- Editor: Ma YJ **L- Editor**: A **E- Editor:** Yan JL

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.113

World J Transplant 2013 December 24; 3(4): 113-118
ISSN 2220-3230 (online)

MINIREVIEWS

Essential concept of transplant immunology for clinical practice

Damodar Kumbala, Rubin Zhang

Damodar Kumbala, Rubin Zhang, Section of Nephrology, Department of Medicine, Tulane University School of Medicine, New Orleans, LA 70112, United States
Author contributions: Kumbala D and Zhang R contributed equally to this manuscript.
Correspondence to: Rubin Zhang, MD, FASN, Professor of Clinical Medicine, Section of Nephrology, Department of Medicine, Tulane University School of Medicine, 1430 Tulane Ave, SL-45, New Orleans, LA 70112, United States. rzhang@tulane.edu
Telephone: +1-504-9881457 Fax: +1-504-9881105
Received: July 9, 2013 Revised: August 24, 2013
Accepted: September 4, 2013
Published online: December 24, 2013

Abstract

Our understanding of transplant immunology has advanced from gross allograft rejection to cellular response and to current molecular level. More sensitive assays have been developed to characterize patient sensitization and to detect pre-existing donor-specific antibodies (DSA) in pre-transplant crossmatch. After a transplant, pre-existing or *de novo* DSA are increasingly monitored to guide clinical management. Therefore, it is important for clinicians to understand the basic concepts and key components of transplant immunology as well as be familiarized with the modern immunological techniques used in kidney transplantation.

Key words: Alloimmune response; Major histocompatibility complex; Human leukocyte antigens; Co-stimulation pathway; Panel reactive antibody; Donor specific antibody; Non-human leukocyte antigens antibody; Crossmatch

Core tip: The alloimmune response is initiated by T-cell recognition of alloantigens through direct or indirect pathways. Three signal models have been established during T-cell activation, which subsequently produces various effector T-cells and antibody production. Sensitive crossmatch is routinely performed before kidney transplant to detect any significant donor-specific antibodies, so that hyperacute rejection can be eliminated. Solid phase based Luminex assay can further characterize human leukocyte antigens antibodies before and after kidney transplant to guide our clinical practice.

Kumbala D, Zhang R. Essential concept of transplant immunology for clinical practice. *World J Transplant* 2013; 3(4): 113-118 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/113.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.113

INTRODUCTION

When a foreign organ, such as a kidney, is transplanted into a non-identical individual of the same species, the organ is called an allograft. The immune response from the recipient to the allograft is termed an alloimmune response, which is initiated by T-cell recognition of alloantigens (commonly known as allorecognition). Allorecognition is the first step of a series of complex events that leads to T-cell activation, antibody production, and allograft rejection[1-3]. This review will summarize the key concepts of transplant immunology and modern immunological assays, which are essential in our clinical practice.

MAJOR HISTOCOMPATIBILITY COMPLEX/HUMAN LEUKOCYTE ANTIGENS MOLECULES

The major histocompatibility complex (MHC) genes code the strongest transplant antigens. In humans, these MHC molecules are called human leukocyte antigens (HLA) and the genetic region is located on the short arm of chromosome 6. Each parent provides a haplotype (a linked set of

MHC genes) to each offspring in Mendelian co-dominant inheritance. There are two classes of MHC or HLA molecules, viz. Class I molecules and Class Ⅱ molecules. Class I molecules (HLA-A, -B, and -C) are composed of a polymorphic heavy chain (α chain, 44 kDa) and a non-polymorphic light chain (β2 microglobulin, 12 kDa). They are expressed on all nucleated cells and generally present endogenous small antigens (typically 9 to 11 amino acids), such as viruses and self-protein fragments, in the context of self-MHC to $CD8^+$ T. Class Ⅱ molecules (HLA-DP, -DQ, and -DR) are composed of a polymorphic α chain (35 kDa) and a β chain (31 kDa). They are constitutively expressed only on professional antigen-presenting cells (APC), including dendritic cells, macrophages, and B-cells. Their expression may be upregulated on epithelial and vascular endothelial cells after exposure to pro-inflammatory cytokines. Class Ⅱ molecules present relatively larger antigens (12 to 28 amino acids), derived from extracellular proteins to $CD4^+$ T-cells[1-4]. The degree of HLA mismatch between donor and recipient plays a role in determining the risk of chronic rejection and graft loss. HLA-A, -B, and -DR (3 pairs, 6 antigens) are traditionally used for typing and matching before kidney or pancreas transplant. HLA-Cw, -DP, and -DQ are now increasingly typed and used in many transplant centers. For kidney transplants, the long-term graft survival is best in HLA-identical living related kidney transplants. The major impact comes from the match of the DR antigen, and the order of importance for HLA match in kidney transplant is DR > B > A[1,3,4].

NON-HLA ANTIGENS/ANTIBODIES

Acute and chronic graft rejection can occur in HLA-identical sibling transplants, indicating the presence of immune response to non-HLA antigens. There are several non-HLA antigens and their antibodies derived from either alloimmunity or autoimmunity have been reported[5,6].

ABO blood group antigens

ABO blood group antigens are not only expressed on red blood cells, but also on vascular endothelial cells and other cells. ABO incompatible organ transplants cause hyperacute rejection due to the presence of the preformed hemagglutinin A and/or B antibody. ABO compatibility between donor and recipient are essential for organ transplant, similar to red blood cell transfusion. Desensitization protocols to remove the preformed hemagglutinin A and/or B from recipient circulation have been used for ABO incompatible kidney transplants[1,7]. The rhesus factor and other red cell antigens are not relevant to organ transplant, as they are not expressed on endothelium.

Minor histocompatibility antigens

Minor histocompatibility antigens (MiHA) are small endogenous peptides that occupy the antigen-binding site of donor MHC molecules. They are generally recognized by $CD8^+$ cytotoxic T-cells in the context of self-MHC, which leads to graft rejection. In bone marrow transplant, MiHA play an important role in graft-*vs*-host disease in patients who have received HLA-matched cells[8]. H-Y MiHA is encoded by the Y chromosome in males and can induce alloimmune response when a male organ is transplanted into a female recipient[9]. MHC class 1 related chain A and B (MICA and MICB) are also expressed on endothelial cells. Antibodies against MICA and/or MICB can cause antibody-mediated rejection (AMR) and graft loss[10].

Other reported antibodies causing graft rejection include anti-angiotensin-2 receptor, anti-glutathione S-transferase T1, and anti-endothelial antibodies[11-13]. Anti-endothelial antibody can be detected by using donor monocytes for crossmatch[13]. Some minor transplant antigens may come from mitochondrial proteins and enzymes. As our knowledge in transplant immunology advances, there will likely be more alloreactive and autoreactive antibodies to uncover.

ALLORECOGNITION PATHWAYS

Allorecognition can occur by one of three pathways: direct, indirect, and semi-direct[14-16]. In the direct pathway, recipient's T-cells recognize intact allogeneic HLAs expressed by donor cells. In the indirect pathway, T-cells recognize peptides derived from donor HLAs presented by recipient APC. In the semi-direct pathway, recipient dendritic cells or other APC acquire intact HLAs from donor cells and present them to recipient T cells. The direct and indirect pathways are well understood in organ transplantation; the semi-direct pathway is not of clinical importance. The direct pathway is very important in the immediate post transplant period. Without appropriate immunosuppression, a strong and effective alloresponse would follow, which is primarily due to the high number of recipient T-cells that will recognize the graft antigens and cause acute cellular rejection. While the indirect pathway of allorecognition may also participate in acute rejection, it is usually predominant in the late onset of rejection, and especially chronic rejection[14-16]. As long as the allograft is present in the host, the recipient APCs can pick up the alloantigen shed from the graft and start alloimmune response. Therefore, maintenance immunosuppression is required for the lifetime of the allograft to prevent late rejection and chronic rejection.

THREE-SIGNAL MODEL OF T-CELL ACTIVATION

T-cell activation is the key process of allograft rejection. T-cells recognize alloantigen through T-cell receptors (TCR). The initiation of intracellular signaling requires additional peptides known as CD3 complex, and the antigen-specific signal (signal 1) is transduced through the TCR-CD3 complex[1-3]. Two signals are needed for complete T-cell activation (Figure 1). The second co-

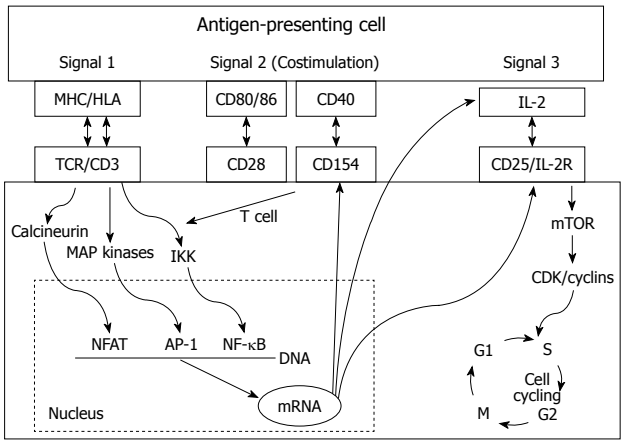

Figure 1 The 3-signal model of T cell activation. MHC: Major histocompatibility complex; HLA: Human leukocyte antigens; IL: Interleukin; TCR: T-cell receptors; NFAT: Nuclear factor of activated T cells; mTOR: Mechanistic target of rapamycin.

stimulatory signal depends on the receptor-ligand interactions between T-cells and APCs (signal 2). Numerous co-stimulatory pathways have been described and blockage of these pathways can lead to antigen-specific inactivation or death of T-cells[17-19]. The best-studied ones are the CD28-B7 and CD154-CD40 pathways. CD28 and CD154 are expressed on T-cells, and their ligands B7 and CD40 are expressed on APCs. CD28 has two ligands, B7-1 (CD80) and B7-2 (CD86). T-cells also express cytotoxic T-lymphocyte associated antigen-4 (CTLA-4), which is homologous to CD28 and has a higher affinity than CD28 to bind B7. However, when CTLA-4 binds B7 (both CD80 and CD86), it produces an inhibitory signal to terminate T-cell response. This unique interaction leads to the clinical development of a fusion protein CTLA-4-Ig (belatacept) as a novel immunosuppressive medication[19]. CD154-CD40 blockages have also been shown to prevent allograft rejection in animal models, including anti-CD154 antibody and molecules that target CD40[18].

The combination of signal 1 and 2 activates three downstream signal transduction pathways: the calcium-calcineurin pathway, the RAS-mitogen activated protein kinase pathway, and the IKK-nuclear factor κB (NF-κB) pathway. These three pathways further activate transcription factors including the nuclear factor of activated T cells, activated protein-1, and NF-κB, respectively. Several new molecules and cytokines including CD25, CD154, interleukin (IL)-2, and IL-15 are subsequently expressed[1-3]. IL-2 and IL-15 deliver growth signals (signal 3) through the mammalian target of rapamycin pathway and phosphoinositide-3-kinase pathway, which subsequently trigger the T-cell cycle and proliferation (Figure 1). The fully activated T-cells undergo clonal expansion and produce a large number of cytokines and effector T-cells, which eventually produce $CD8^+$ T-cell mediated cytotoxicity, help macrophage-induced delayed type hypersensitivity response (by $CD4^+$Th1), and help B cells for antibody production (by $CD4^+$Th2). A subset of activated T-cells becomes the alloantigen-specific memory T-cells[20,21].

B LYMPHOCYTES

B-cells express clonally restricted antigen-specific receptors as immunoglobulins on their surfaces. When these receptors bind donor HLA antigens in the context of assistance from helper T-cells ($CD4^+$Th2), B-cells are activated. They then divide, differentiate, and become plasma cells to secret antibodies. Some activated B-cells become memory B-cells[22-24]. The helper T-cells may facilitate B-cell activation either through intimate membrane contact involving a variety of receptors and ligands (such as CD40:CD154) or through the secreted soluble cytokines (such as IL-4)[18,23,24]. These HLA antibodies bind antigens and can cause graft injury either by activating the complement cascade [complement-dependent cytotoxicity (CDC)] or *via* Fc receptor on natural killer (NK) cells, neutrophils, and eosinophils (antibody-dependent cellular cytotoxicity)[1,14]. In addition producing antibodies, B-cells are also APCs. B-cells can present allograft-derived antigens to T-cells for T cell activation through the indirect pathway of allorecognition[2-4].

INNATE AND ADAPTIVE IMMUNE RESPONSES IN GRAFT REJECTION

Innate immunity refers to the nonspecific natural immune system that involves macrophages, neutrophils, NK cells, cytokines, toll-like receptors, and complement components[25]. Alloimmune is an adaptive immunity that involves recognition of alloantigen and confers antigen specificity and memory by T and B cells as discussed above. However, alloimmune response not only produces specific effector T cells and antibodies, but also secretes chemokines and cytokines, which recruit components of the innate immune system, such as complement activation and leukocyte migration from the circulation into a site of inflammation[1-4]. On the other hand, ischemic injury of the allograft initially activates the innate immune response, which leads to increased antigen presentation to T-cells by up-regulating the expression of class Ⅱ HLAs, adhesion molecules, and cytokines[2-4]. Therefore, the innate and adaptive immune responses are closely interrelated and both play important roles in allograft rejection and rejection-associated tissue damage.

SENSITIZATION AND PANEL REACTIVE ANTIBODY

Human sensitization is defined by the presence of antibodies in the recipient's blood against a panel of selected HLA antigens representing donor population. It is reported as the percent panel reactive antibody (PRA). PRA estimates the likelihood of positive crossmatches to potential donors[1,14]. The higher the PRA level, the lower the chance of receiving a compatible kidney and longer the waiting time on the kidney waitlist, previous exposure to HLA antigens. Sensitization is caused by previous ex-

posure to HLA antigens, usually through previous organ transplant(s), pregnancy or blood transfusion particularly relevant is the exposure of women to their partner's HLA during pregnancy. This results in direct sensitization against the partner, potentially making the partner and/or their child an unsuitable donor. The percent PRA in an individual patient may vary from one testing date to another secondary to either a change in antibody titers, or a change in the usage of HLA antigens in the assay. The technology of PRA assay has advanced from the initial CDC assay, to the enzyme-linked immunoabsorption (ELISA), to the current multiplexed particle-based flow cytometry (Luminex). Single antigen beads are increasingly used to characterize the preformed HLA antibodies before transplant as well as any *de novo* development of HLA antibodies (donor-specific antibodies, DSA) after transplant[1,26].

CROSSMATCH AND DSA

Solid phase based ELISA or Luminex assay can detect and characterize the preformed HLA antibodies in an individual patient. The corresponding antigens are considered unacceptable for that patient, and in the unites states of America (United States), they are listed into the United Network of Organ Sharing database. A patient will not be offered a kidney from the deceased donor who expresses an unacceptable HLA antigen (positive virtual crossmatch). Only those patients whose HLA antibodies are not donor directed will appear on the match run (negative virtual crossmatch). Such "virtual crossmatch" can improve efficiency of organ allocation by decreasing the risk of positive crossmatch before transplant[26]. When a potential donor is identified, a final crossmatch with fresh serum from recipient and lymphocytes from donor has to be performed to rule out any preformed DSA, which can produce hyperacute AMR. The final crossmatch must be negative to proceed with kidney transplantation. The two commonly used tests for evaluation of kidney transplant eligibility are CDC crossmatch and flow cytometry crossmatch (FCXM). The choice of which crossmatch test to perform remains a controversial issue. Individual transplant programs, according to center experience and availability, usually determine it.

T-cells express HLA class Ⅰ antigens only, while B-cells express both HLA class Ⅰ and class Ⅱ antigens. Furthermore, B-cells express HLA class Ⅰ antigens at quantitatively greater level than on T-cells. T-cell positive crossmatch is considered as true and significant sensitization with DSA against HLA class Ⅰ antigens. T-cell negative/B-cell positive crossmatches may represent either HLA class Ⅱ antibodies or low titers of HLA class Ⅰ antibodies. T-cell positive/B-cell negative results are likely due to presence of non-HLA antibodies[1,3].

CDC CROSSMATCH

The donor lymphocytes (T-cells, B-cells, or mixed) are isolated from blood or lymph nodes, and placed in wells. The recipient serum is then added along with rabbit complement. The cytotoxicity is determined by counting the lyses of lymphocytes compared with a control. It is usually modified by addition of antihuman globulin to increase the sensitivity (AHG-CDC), as antihuman globulin can induce cross-linking of antibodies and increase the visual cytotoxicity. If the initial CDC crossmatch is positive, it will be repeated with the addition of dithiothreitol (DTT), which reduces the disulfide bonds of immunoglobulin (Ig)M if it is present. Initial positive and repeated DTT positive tests indicate the presence of DSA of IgG rather than IgM. IgM antibodes are generally not considered to be real sensitization. Kidney transplantation should not proceed if there is evidence of a positive crossmatch secondary to a cytotoxic IgG anti-HLA antibody. However, there are various desensitization protocols that can be used to remove the preformed DSA to achieve negative final crossmatch for HLA incompatible transplants if a living donor is involved[27-31].

FCXM

Donor T and B-lymphocytes are isolated and mixed with recipient serum. A fluorescence labeled antihuman IgG is then added. The cells that bind any recipient antibodies are stained with fluorescence labeled antihuman IgG and cause the channel shifts in fluorescent intensity. FCXM is much more sensitive than CDC or AHG-CDC in detecting low level of antibodies. Non-cytotoxic antibodies can also be detected with FCXM since it does not depend on the complement activation of antibody. The significance of non-complement activating or non-cytotoxic antibodies *in-vivo* is unclear. Single antigen bead (Luminex) can be used to further characterize any DSA presence and to determine if the DSA is responsible for the channel shift in the flow crossmatch[1-3].

Again, these two crossmatches differ in the degree of sensitivity. Conservative transplant programs may choose sensitive FCXM, which will significantly reduce the incidence of post-transplant AMR. However, it may also be too sensitive in that clinically irrelevant antibodies are detected. Consequently, some viable transplant opportunities are potentially lost. Crossmatch tests can also be performed with the recipient's previous sera. The scenario of current sera negative, historical sera positive suggests previous antibodies may have waned in titer. But the specific memory B-cells could rapidly expand and produce the antibodies when re-exposed to the specific alloantigen. Although this is not considered as a contraindication for transplantation, it does increase the risk of AMR after transplant. Close monitoring of DSA titer and more immunosuppression is usually recommended.

CONCLUSION

The alloimmune response is initiated by T-cell recognition of alloantigens through direct or indirect pathways.

Three signal models have been established during T-cell activation, which subsequently produces various effector T-cells and antibody production. Sensitive crossmatch is routinely performed before kidney transplant to detect any significant DSA, so that hyperacute rejection can be eliminated. Solid phase based Luminex assay can further characterize HLA antibodies before and after kidney transplant to guide our clinical practice. In addition to the traditional anti-HLA antibodies, alloreactive and autoreactive antibodies against non-HLA antigens have now been increasingly recognized to play an important role in humoral rejection of allograft.

ACKNOWLEDGMENTS

We thank Michael Yatsevitch for assisting us in preparing the figure.

REFERENCES

1 **Klein C**, Brennan DC. HLA and ABO sensitization and desensitization in renal transplantation. UpToDate [Internet], 2013. Available from: URL: http://www.uptodate.com/contents/hla-and-abo-sensitization-and-desensitization-in-renal-transplantation

2 **Halloran PF**. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715-2729 [PMID: 15616206 DOI: 10.1056/NEJMra033540]

3 **Danovitch GM**. Handbook of kidney transplantation.4th ed. Philadelphia: Lippincott Williams and Wilkins, 2005: 23-71

4 **Lakkis FG**, Sayegh MH. Memory T cells: a hurdle to immunologic tolerance. *J Am Soc Nephrol* 2003; **14**: 2402-2410 [PMID: 12937320]

5 **Dinavahi R**, George A, Tretin A, Akalin E, Ames S, Bromberg JS, Deboccardo G, Dipaola N, Lerner SM, Mehrotra A, Murphy BT, Nadasdy T, Paz-Artal E, Salomon DR, Schröppel B, Sehgal V, Sachidanandam R, Heeger PS. Antibodies reactive to non-HLA antigens in transplant glomerulopathy. *J Am Soc Nephrol* 2011; **22**: 1168-1178 [PMID: 21566057]

6 **Porcheray F**, DeVito J, Yeap BY, Xue L, Dargon I, Paine R, Girouard TC, Saidman SL, Colvin RB, Wong W, Zorn E. Chronic humoral rejection of human kidney allografts associates with broad autoantibody responses. *Transplantation* 2010; **89**: 1239-1246 [PMID: 20445487 DOI: 10.1097/TP.0b013e3181d72091]

7 **Montgomery RA**. Renal transplantation across HLA and ABO antibody barriers: integrating paired donation into desensitization protocols. *Am J Transplant* 2010; **10**: 449-457 [PMID: 20121749 DOI: 10.1111/j.1600-6143.2009.03001.x]

8 **Warren EH**, Greenberg PD, Riddell SR. Cytotoxic T-lymphocyte-defined human minor histocompatibility antigens with a restricted tissue distribution. *Blood* 1998; **91**: 2197-2207 [PMID: 9490709]

9 **Scott DM**, Ehrmann IE, Ellis PS, Chandler PR, Simpson E. Why do some females reject males? The molecular basis for male-specific graft rejection. *J Mol Med* (Berl) 1997; **75**: 103-114 [PMID: 9083928 DOI: 10.1007/s001090050095]

10 **Hankey KG**, Drachenberg CB, Papadimitriou JC, Klassen DK, Philosophe B, Bartlett ST, Groh V, Spies T, Mann DL. MIC expression in renal and pancreatic allografts. *Transplantation* 2002; **73**: 304-306 [PMID: 11821751 DOI: 10.1097/00007890-200201270-00029]

11 **Dragun D**, Müller DN, Bräsen JH, Fritsche L, Nieminen-Kelhä M, Dechend R, Kintscher U, Rudolph B, Hoebeke J, Eckert D, Mazak I, Plehm R, Schönemann C, Unger T, Budde K, Neumayer HH, Luft FC, Wallukat G. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med* 2005; **352**: 558-569 [PMID: 15703421 DOI: 10.1056/NEJMoa035717]

12 **Aguilera I**, Alvarez-Marquez A, Gentil MA, Fernandez-Alonso J, Fijo J, Saez C, Wichmann I, Nuñez-Roldan A. Anti-glutathione S-transferase T1 antibody-mediated rejection in C4d-positive renal allograft recipients. *Nephrol Dial Transplant* 2008; **23**: 2393-2398 [PMID: 18308775]

13 **Sun Q**, Cheng Z, Cheng D, Chen J, Ji S, Wen J, Zheng C, Liu Z. De novo development of circulating anti-endothelial cell antibodies rather than pre-existing antibodies is associated with post-transplant allograft rejection. *Kidney Int* 2011; **79**: 655-662 [PMID: 20980975 DOI: 10.1038/ki.2010.437]

14 **Khan IE**, Zhang R, Simon EE, Hamm LL. The allo-immunological injury in chronic allograft nephropathy. In: Göőz M, eidtor. Chronic kidney disease. Shanghai: InTech, 2012: 401-414

15 **Liu Z**, Colovai AI, Tugulea S, Reed EF, Fisher PE, Mancini D, Rose EA, Cortesini R, Michler RE, Suciu-Foca N. Indirect recognition of donor HLA-DR peptides in organ allograft rejection. *J Clin Invest* 1996; **98**: 1150-1157 [PMID: 8787678 DOI: 10.1172/JCI118898]

16 **Vella JP**, Spadafora-Ferreira M, Murphy B, Alexander SI, Harmon W, Carpenter CB, Sayegh MH. Indirect allorecognition of major histocompatibility complex allopeptides in human renal transplant recipients with chronic graft dysfunction. *Transplantation* 1997; **64**: 795-800 [PMID: 9326400 DOI: 10.1097/00007890-199709270-00001]

17 **Durrbach A**, Francois H, Jacquet A, Beaudreuil S, Charpentier B. Co-signals in organ transplantation. *Curr Opin Organ Transplant* 2010; **15**: 474-480 [PMID: 20631615]

18 **Denton MD**, Reul RM, Dharnidharka VR, Fang JC, Ganz P, Briscoe DM. Central role for CD40/CD40 ligand (CD154) interactions in transplant rejection. *Pediatr Transplant* 1998; **2**: 6-15 [PMID: 10084754]

19 **Larsen CP**, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobert E, Anderson D, Cowan S, Price K, Naemura J, Emswiler J, Greene J, Turk LA, Bajorath J, Townsend R, Hagerty D, Linsley PS, Peach RJ. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant* 2005; **5**: 443-453 [PMID: 15707398 DOI: 10.1111/j.1600-6143.2005.00749.x]

20 **Fiorentino L**, Austen D, Pravtcheva D, Ruddle FH, Brownell E. Assignment of the interleukin-2 locus to mouse chromosome 3. *Genomics* 1989; **5**: 651-653 [PMID: 2613246]

21 **Tsaur I**, Gasser M, Aviles B, Lutz J, Lutz L, Grimm M, Lange V, Lopau K, Heemann U, Germer CT, Chandraker A, Waaga-Gasser AM. Donor antigen-specific regulatory T-cell function affects outcome in kidney transplant recipients. *Kidney Int* 2011; **79**: 1005-1012 [PMID: 21270769 DOI: 10.1038/ki.2010.533]

22 **Mills DM**, Cambier JC. B lymphocyte activation during cognate interactions with CD4+ T lymphocytes: molecular dynamics and immunologic consequences. *Semin Immunol* 2003; **15**: 325-329 [PMID: 15001171 DOI: 10.1016/j.smim.2003.09.004]

23 **Richards S**, Watanabe C, Santos L, Craxton A, Clark EA. Regulation of B-cell entry into the cell cycle. *Immunol Rev* 2008; **224**: 183-200 [PMID: 18759927 DOI: 10.1111/j.1600-065X.2008.00652.x]

24 **Gatto D**, Martin SW, Bessa J, Pellicioli E, Saudan P, Hinton HJ, Bachmann MF. Regulation of memory antibody levels: the role of persisting antigen versus plasma cell life span. *J Immunol* 2007; **178**: 67-76 [PMID: 17182541]

25 **Takeuchi O**, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010; **140**: 805-820 [PMID: 20303872 DOI: 10.1016/j.cell.2010.01.022]

26 **Ren Q**, Paramesh A, Yau CL, Killackey M, Slakey D, Florman S, Buell J, Alper B, Simon E, Hamm LL, Zhang R. Long-term outcome of highly sensitized African Ameri-

can patients transplanted with deceased donor kidneys. *Transpl Int* 2011; **24**: 259-265 [PMID: 21091553 DOI: 10.1111/j.1432-2277.2010.01188.x]

27 **Lorenz M**, Regele H, Schillinger M, Kletzmayr J, Haidbauer B, Derfler K, Druml W, Böhmig GA. Peritransplant immunoadsorption: a strategy enabling transplantation in highly sensitized crossmatch-positive cadaveric kidney allograft recipients. *Transplantation* 2005; **79**: 696-701 [PMID: 15785376 DOI: 10.1097/01.TP.0000148732.26761.FA]

28 **Montgomery RA**, Lonze BE, King KE, Kraus ES, Kucirka LM, Locke JE, Warren DS, Simpkins CE, Dagher NN, Singer AL, Zachary AA, Segev DL. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011; **365**: 318-326 [PMID: 21793744 DOI: 10.1056/NEJMoa1012376]

29 **Stegall MD**, Gloor J, Winters JL, Moore SB, Degoey S. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. *Am J Transplant* 2006; **6**: 346-351 [PMID: 16426319 DOI: 10.1111/j.1600-6143.2005.01178.x]

30 **Vo AA**, Lukovsky M, Toyoda M, Wang J, Reinsmoen NL, Lai CH, Peng A, Villicana R, Jordan SC. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med* 2008; **359**: 242-251 [PMID: 18635429 DOI: 10.1056/NEJMoa0707894]

31 **Warren DS**, Zachary AA, Sonnenday CJ, King KE, Cooper M, Ratner LE, Shirey RS, Haas M, Leffell MS, Montgomery RA. Successful renal transplantation across simultaneous ABO incompatible and positive crossmatch barriers. *Am J Transplant* 2004; **4**: 561-568 [PMID: 15023148 DOI: 10.1111/j.1600-6143.2004.00364.x]

P- Reviewers: Rodriguez DC, Sun XY **S- Editor**: Gou SX
L- Editor: A **E- Editor**: Zheng XM

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.119

World J Transplant 2013 December 24; 3(4): 119-126
ISSN 2220-3230 (online)

MINIREVIEWS

Preclinical stem cell therapy in Chagas Disease: Perspectives for future research

Katherine Athayde Teixeira de Carvalho, Eltyeb Abdelwahid, Reginaldo Justino Ferreira, Ana Carolina Irioda, Luiz Cesar Guarita-Souza

Katherine Athayde Teixeira de Carvalho, Ana Carolina Irioda, Cell Therapy and Biotechnology in Regenerative Medicine Research Group, Pequeno Príncipe Faculty, Pelé Pequeno Príncipe Institute, Curitiba 80250-200, Paraná, Brazil
Katherine Athayde Teixeira de Carvalho, Reginaldo Justino Ferreira, Bioprocess Engineering and Biotechnology Department, Federal University of Paraná, Curitiba 81531-970, Paraná, Brazil
Eltyeb Abdelwahid, CBRC, Massachusetts General Hospital/Harvard Medical School, Charlestown, Ma 02129, United States
Reginaldo Justino Ferreira, Federal University of Technology, Curitiba 80230-901, Paraná, Brazil
Luiz Cesar Guarita-Souza, Experimental Laboratory of Institute of Biological and Health Sciences of Pontifical Catholic University of Parana, Curitiba 80215-901, Paraná, Brazil
Author contributions: de Carvalho KAT proposed the review, analyzed the data; Irioda AC collected the data; de Carvalho KAT, Abdelwahid E and Guarita-Souza LC wrote the manuscript; Ferreira RJ performed the references; de Carvalho KAT edited the manuscript.
Correspondence to: Katherine Athayde Teixeira de Carvalho, Professor, MD, PhD, Cell Therapy and Biotechnology in Regenerative Medicine Research Group, Pequeno Príncipe Faculty, Pelé Pequeno Príncipe Research Institute, Av. Siva Jardim 1632, Curitiba 80250-200, Paraná, Brazil. katherinecarv@gmail.com
Telephone: +55-41-33101719 Fax: +55-41-33221446
Received: August 29, 2013 Revised: September 16, 2013
Accepted: October 18, 2013
Published online: December 24, 2013

Abstract

Chagas cardiomyopathy still remains a challenging problem that is responsible for high morbidity and mortality in Central and Latin America. Chagas disease disrupts blood microcirculation via various autoimmune mechanisms, causing loss of cardiomyocytes and severe impairment of heart function. Different cell types and delivery approaches in Chagas Disease have been studied in both preclinical models and clinical trials. The main objective of this article is to clarify the reasons why the benefits that have been seen with cell therapy in preclinical models fail to translate to the clinical setting. This can be explained by crucial differences between the cellular types and pathophysiological mechanisms of the disease, as well as the differences between human patients and animal models. We discuss examples that demonstrate how the results from preclinical trials might have overestimated the efficacy of myocardial regeneration therapies. Future research should focus, not only on studying the best cell type to use but, very importantly, understanding the levels of safety and cellular interaction that can elicit efficient therapeutic effects in human tissue. Addressing the challenges associated with future research may ensure the success of stem cell therapy in improving preclinical models and the treatment of Chagas disease.

Key words: Chagas Disease; Preclinical; Stem cell; Therapy; Co-cultured; Translation; Pathophysiologie; Myoblasts

Core tip: The manuscript discusses examples that demonstrate how the results from preclinical trials might have overestimated the efficacy of myocardial regeneration with cell therapies, particularly in Chagas Disease and addressing the challenges associated with future research. The failure of cell therapy can be explained by crucial differences between the cellular types and pathophysiological mechanisms of the disease, as well as the differences between human patients and animal models.

de Carvalho KAT, Abdelwahid E, Ferreira RJ, Irioda AC, Guarita-Souza LC. Preclinical stem cell therapy in Chagas

Disease: Perspectives for future research. *World J Transplant* 2013; 3(4): 119-126 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/119.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.119

INTRODUCTION

Chronic Chagas Disease is the most common form of cardiomyopathy in Central and South America and is one of the leading causes of death from cardiovascular disease in endemic areas. To date, there is no effective treatment for this disease apart from pharmacological treatment. Patients as described above may derive some benefits from beta-blockers, inhibitors of angiotensin conversion enzyme and diuretics[1].

The only effective treatment available for individuals who develop a more severe disease, such as heart failure due to Chagas Disease, is total organ transplantation, *i.e.,* heart transplantation. This procedure is limited due to its high cost, and the scarcity of donated organs; the immune suppressor drugs used in this situation can also reactivate the disease.

On the other hand, regenerative medicine has emerged with new perspectives on cell-based therapy to add to the established drug therapy of Chagasic cardiomyopathy in order to prevent heart failure progressing or to prolong and improve the quality of life of patients.

However, these possibilities should be viewed with caution in light of the pathophysiological aspects of cardiomyopathy.

There are currently various cell types that can be used in cell therapy: isolated cells, or in combinations, and associated (or not) with the arrays. Another important variable is the manner in which cells are administered: catheterisation, epicardial or intramyocardial injection. In this context, preclinical research is fundamental for better identification of the type of cell therapy that is functionally effective for translation to humans as well as for identifying the therapeutic availability and risks involved. Cell therapy should be both feasible and safe.

The aim of cell therapy in relation to the heart, independently of ischemic or Chagas Disease, is to obtain myocardial regeneration and to improve heart function by cell-replacement therapy, as well as to reverse the geometric remodeling of ventricular cavities.

Consensus on the most appropriate form of cell therapy should be based on the best functional outcomes in preclinical studies. Animal models are important tools in experimental medical science to better understand the pathogenesis of human disease and to test therapeutic approaches.

Many reasons have been proposed for the failures of clinical trials, including the choice of cellular type for therapy. In this article we discuss the selection of preclinical models because this is one of the main reasons why clinical translation has been unsuccessful thus far. This issue has received little attention, but it may have had dramatic implications for the expectations of clinical trials. We highlight crucial differences between cellular types and pathophysiological mechanisms of the disease, as well as the differences between human patients and animal models, with regards to a better understanding of the results obtained so far and to reflect on the perspectives for future research. We use examples to demonstrate why the results from preclinical trials might have overestimated the efficacy of the myocardial regeneration therapies that have been developed to date. We also suggest ways in which currently available animal models of Chagas could be translated for human use and also offer advice on how to work with existing models to avoid overestimating the efficacy of single bone marrow cell therapies.

All models have advantages and disadvantages and the choice of stem cell therapy model should be based on the specific pathophysiological mechanisms of the disease; nevertheless, for myocardial regeneration, cell therapy requires the development of myogenesis, for contraction as well as angiogenesis, independent of the disease pathophysiology, because the striated muscle, like the myocardial and its cardiomyocytes (CMCs), needs nutrition.

PATHOPHYSIOLOGICAL MECHANISMS

For the development of therapeutics based on stem cells in Chagas Disease, some authors have considered approaches or tests that are similar to those performed in ischemic cardiomyopathies, not taking into account the fundamental differences between the pathophysiologies. This explains why the results obtained in humans differ from preclinical results; the intracoronary injection of autologous mononuclear cells in humans has not improved left ventricular function or the quality of life of patients with chronic Chagas cardiomyopathy. These results were different from those obtained in a preclinical model[2].

The death of CMCs may be due to many factors, such as myocardial infarction or other causes, like Chagas cardiomyopathy, which cause fibrosis in the remodeling of the left ventricle due to the fact that adult CMCs have only a limited capacity to regenerate and are insufficient to resolve heart tissue injury[3].

In myocardial infarction there is a loss of cardiac vascular supply, accompanied by pro-inflammatory events with increased production of 6-interleukin and tumor necrosis factor, leading to cellular necrosis, loss of CMCs in the heart region and heart dysfunction[4,5].

The pathophysiology of the chronic form of Chagas cardiomyopathy is still not very clear. Among the various mechanisms are: a parasite-mediated tissue destruction, denervation plexus infarction, platelet aggregation, and intravascular lesion tissue mediated autoimmune mechanisms. The disproportion of parasites suggests relationships with autoimmune mechanisms[4-9].

In Chagas Disease, infection by *Trypanosoma cruzi* (*T. cruzi*) causes a generalised inflammatory vascular disease,

characterised by the presence of vasospasm, a reduction in blood flow, focal ischemia, thrombosis, increased platelet aggregation, and higher levels of thromboxane A2 and endothelin-1. Endothelial cell infection by the parasite increases with the synthesis of endothelin-1, which participates in the vasospasm of the coronary microcirculation[5].

In summary, myocardial Chagas Disease is a diffuse lesion due to the interrupted microcirculation of blood vessel supplies mediated by autoimmune mechanisms, causing the loss of CMCs and remodeling process with the impairment of heart function.

CELLULAR TYPES

To obtain myocardium regeneration, various cell types were evaluated; undifferentiated cells such as stem cells, and differentiated cells like CMCs or myoblasts. However, not all cellular types have been evaluated in Chagas Disease.

As regards undifferentiated cells, there are embryonic stem cells (ESCs) or adult stem cells (ASCs). The ASCs can be of diverse origin and can include: bone marrow-derived stem cells; bone marrow mononuclear cells (known as hematopoietic stem cells) such as $CD45^+/CD34^-$; hematopoietic-derived mesenchymal stem cells (hMSC, known as bone marrow mesenchymal stem cells) such as $CD45^-/CD34^-$; adipose-derived stem cells; mesenchymal fraction such as $CD45^-/CD34^+/CD105^+/CD90^+/CD73^+$; umbilical cord blood-derived stem cells; mononuclear cells such as $CD45^+/CD34^-$ and mesenchymal cells $CD45^-/CD34^-$; and induced pluripotent stem cells such as octamer-binding transcription factor 4+[10,11].

There are only three preclinical models that have been tested for cell therapy in Chagas cardiomyopathy: (1) bone marrow mononuclear stem cells; (2) co-cultured cells; myoblasts such as $CD56^+$ with bone marrow mesenchymal stem cells; and (3) isolated bone marrow mesenchymal stem cells[6-9]. The other cellular types are still only a theoretical approach[12].

ASCS

Bone marrow-derived stem cells

Bone marrow was the first source of stem cells for application in various preclinical models for many diseases, including heart disease. This followed extensive clinical experience with these cells in their use for the treatment of onco-hematological diseases. Cells obtained from bone marrow have many advantages; they are easy to obtain and they do not require cultivation (reduced risk of contamination and for transformation), which allows the possibility of autologous therapy without the need for immune suppressor drugs and their adverse effects.

These cells can be obtained by puncture of the iliac crest bone marrow, or from peripheral blood by apheresis with the aid of granulocyte stimulating factor, which mobilises the cells of the bone marrow into peripheral blood. In addition to these advantages, there is increased knowledge about the immune phenotypic characterisation and the quantification of these cells by flow cytometric analysis, ensuring standardisation of protocols.

To test the efficacy of cellular therapy with stem cells from bone marrow in the Chagasic cardiomyopathy, the experimental model of inbred mice chronically infected with the Colombian strain of *T. cruzi* has been used, which caused the development of Chagasic cardiomyopathy in these animals. Mononuclear cells from bone marrow were obtained by lavage of the femurs of the animals and they were injected intravenously into mice during the chronic infection. The degree of inflammation and fibrosis in the heart was assessed after euthanasia of the treated and control animals and the histological sections of the heart were compared[13].

The results of the aforementioned research demonstrated that treated mice showed a significant improvement in myocarditis 2 mo after transplantation when compared to untreated controls. This was explained by the authors as the result of an increase in apoptosis in the inflammatory cells, which caused the loss of CMCs. A decrease in the area of fibrosis was also demonstrated, suggesting that this is a reversible process[13,14].

Another strategy to better understand the action of mesenchymal stem cells (MSC) from bone marrow (BM) in myocardium repair was recently carried out by Jasmin *et al*[15]. This study demonstrated the beneficial effects of MSC therapy in mice model of Chagas Disease, arising from an indirect action of the cells in the heart, rather than a direct action due to the incorporation of large numbers of transplanted bone marrow mesenchymal stem cells (BMMSC) into working myocardium. The authors used cell tracking, following the labelling of MSCs with nanoparticles to investigate the migration of transplanted BMMSCs to the heart.

Co-cultured model of BMMSC and myoblasts

Carvalho *et al*[6] proposed the autologous transplantation of the co-cultured BMMSC and myoblasts for myocardial regeneration in Wistar rats. Their first report proposed the cultivation of both cellular types in a co-cultured model to obtain cells capable of promoting angiogenesis by BMMSC and myogenesis by myoblasts for ischemic myocardium, and at the same time to reduce costs and cultivation time. This co-cultured model had been tested previously in myocardial infarction and compared with myoblasts, co-cultured cells and control. The control was operated animal and injected the medium (Dulbecco's Modified Eagle Medium-DMEN) without cells as sham. The results demonstrated an improvement in left ventricular ejection fraction (LVEF) in both the groups that received cells, with additional results in histopathological analysis-the presence of angiogenesis and myogenesis in the group that received the co-cultured cells[6,16,17].

This model was subsequently transferred for preclinical Chagas cardiomyopathy. In this particular study,

80 rats were inoculated with a single intraperitoneal injection of 150000 trypomastigotes of *T. cruzi*. An ELISA test for Chagas Disease was performed in a sample of the animals. After 8 mo of inoculation, they underwent transthoracic echocardiography for baseline evaluation of heart function. Of the 15 animals that developed ventricular dysfunction with LVEF, less than 35% were randomly submitted to treatment. The incidence obtained of animals with Chagas cardiomyopathy was similar to that which has been described in humans[6,16-18].

Seven animals underwent autologous co-cultured cell transplantation by direct injection ($\times 10^6$ co-cultured product) in the epicardial in open surgery, *vs* eight animals in the control group, which was followed a natural evolution (not sham). At one month after treatment, all the animals were submitted to transthoracic echocardiography. The product of the co-cultured cells was identified by immunocytochemistry assay for identification; antibody anti-fast-myosin for skeletal muscle cells demonstrated by FITC immunofluorescence, and antibody anti-Ⅷ factor for new vessels by demonstrated immunoperoxidase[16,17].

One month after transplantation, in the echocardiographic functional analysis the group of Chagas Disease that had received co-cultured cells demonstrated significantly improved LVEF, 31.10 ± 5.78 to 53.37 ± 5.84 *vs* natural evolution ($P < 0.001$). There was also negative remodelling, which was demonstrated by left ventricular-end diastolic volume (LVEDV), co-cultured cells transplant group: 0.83 ± 0.08 to 0.64 ± 0.16 ($P \leqslant 0.005$) *vs* natural evolution, 0.68 ± 0.12 to 0.72 ± 0.16. Histopathological analysis demonstrated the presence of skeletal muscle cells, like myotube (immature skeletal muscle), and new vessels in hosted myocardial[16,17].

This model demonstrates that negative left ventricular remodelling, as well as reducing the progression of heart failure, may stabilise alterations in the biology of cardiomyocytes, (for example, hypertrophy) and maintain the contractile performance of myocardium[16,17]. On the other hand, Hagège *et al*[19], in relation to human ischemic cardiomyopathy, only transplanted myoblasts. In patients with severe heart failure, the clinical status and Ejection Fraction of patients improves in a stable manner over time, with a strikingly low incidence of hospitalisations for heart failure (0.13/patient-years) and arrhythmic risk can be controlled by medical therapy and/or on-request automatic cardiac defibrillator implantation. In this preclinical model, arrhythmia was not observed[18].

The co-cultured model seems to offer the promise of a treatment that adds to adjuvant therapy for Chagasic cardiomyopathy in patients and the bioprocess of this co-culture has been translated for use in humans; however, this model has not yet been evaluated in human Chagas Disease. Permission has been granted to test in Ⅰ Phase Human by the Brazilian Human Research Ethics Committee, and testing should start soon.

PERSPECTIVES FOR FUTURE

Human embryonic stem cells for cell therapy

In contrast to ASCs, ESCs have the potential to differentiate between the tissue derivatives of all three embryonic germ layers and therefore they are termed pluripotent. CMCs have been obtained from all three types of murine embryo-derived stem cells: embryonic carcinoma (EC), embryonic stem (ES), and embryonic germ (EG) cells. We focus our attention on ESCs due to their potential clinical application. Human embryonic stem cells (hESC) lines, isolated from the inner cell mass of embryos, can be propagated continuously in the undifferentiated state when grown on top of a mouse embryonic fibroblast feeder layer. When removed from these conditions and grown in suspension, they begin to generate three-dimensional differentiating cell aggregates, termed embryoid bodies[19].

Given the versatility of hESCs, and the possibility of obtaining beating CMCs from them, they appear to be the main candidate for cell-based applications for cardiac repair. In fact, hESCs apparently fulfill most, if not all, of the properties of an ideal donor cell line[20].

A possible strategy for cell-replacement therapy could be to initially allow the spontaneous differentiation of ESCs into multiple lineages *in vitro*, followed by selective purification of the cardiomyogenic lineage isolated from embryoid bodies. On this issue, Kehat *et al*[21,22] showed that transplanted hESC-derived CMCs substituted damaged pacemaker cells in a swine model of atrioventricular block, and were responsible for eliciting an ectopic rhythm compatible with the animal's survival. Their results provide compelling evidence that this type of graft integrates electromechanically within the recipient tissue, as discussed by Menasché[23].

Nevertheless, the following obstacles still remain unsolved: (1) The yield of CMC production has to be dramatically improved. It is fundamental to work on the "ideal" culture conditions for CMC differentiation. Unfortunately, the definition of strategies useful for this aim is not easy. The inherent differences between hESCs and their murine counterpart necessitate the obligatory use of hESCs as a model; laws and ethical considerations place strong limitations on what can be done. A further complication is represented by differences between the various protocols[23,24]; (2) hESC lines and their characterisation which, to date, has been unsystematic[25-32]. It appears that each hESC line possesses a unique expression signature and a distinct cardiomyogenic potential[33]. Stimuli useful for directing hESCs through the cardiac lineage are still only being investigated[32-34]. A methodic, combinatorial approach, using various stimuli (trans-stimuli, extra-cellular matrices, co-culture, physical stimuli) could be the best way of directing the differentiation of stem cells *in vitro* in a cardiac stringent-specific way. This speculation is supported by the fact that, when in their natural milieu, cardiomyogenic differentiation of stem cells probably involves multiple signalling pathways.

This may be mimicked *in vitro* with a combination of various methods that achieve a synergistic effect. In fact, *in vitro*-derived, prevascularised scaffold-free cardiac tissue patches from co-culture of CMCs, endothelial cells and fibroblasts were found to greatly improve cell viability, post-transplantation[34]; (3) Culture media. For clinical applications, it is imperative to develop well-defined and efficient *in vitro* protocols for the cardiomyogenic differentiation of stem cells, which use chemically defined culture media supplemented with recombinant cytokines and growth factors. The main drawback of the current xen support system is the risk of cross-transfer of animal pathogens that might hamper future clinical applications. It was recently shown that non-human sialic acid Neu5Gc (against which many humans have circulating antibodies) was incorporated into hES cells grown on mouse feeder layers[35]. The use of human plasma-derived serum, and the development of a serum-free support system and animal-free feeder layer consisting of human fetal fibroblasts and adult epithelial cells or foreskin cells, may provide an appropriate solution to these risks. Nevertheless, *in vitro* up-scaling of clinical grade cell products that are essentially free of xenogenic products, in compliance with good manufacturing practice, remains a significant hurdle[36-40]; (4) Competency of derived CMCs in terms of excitation-contraction coupling. Another important issue is to what extent these cells can be considered mature CMCs as regards excitation-contraction coupling. Indeed, heterogenous electrophysiological properties have been demonstrated in CMCs derived from separate differentiation methods within the same group[40]. This question cannot be accurately answered at the moment since the differentiation procedure has not been efficiently or even minimally standardised. However, some data provide fairly convincing evidence that hESCs can integrate electrically with the recipient myocardium, suggesting that they are capable of contributing to the augmentation of pump function following injury[20]; (5) Immune rejection has to be blocked. Upon differentiation, ES cells express molecules of the major histocompatibility complex (MHC), in particular MHC Ⅰ, while MHC Ⅱ expression levels are low or absent[41]. Thus, decreasing the expression of MHC Ⅰ by genetic modification could improve immunologic tolerance. Alternatively, minimal but targeted conditioning of CD4 and CD8 T-cells may be an option to promote tolerance of embryonic stem cell-derived tissues[42]; and (6) Tumorigenicity may be a problem, even when terminally differentiated CMCs are used for cell replacement. The implantation of undifferentiated ES cells leads to the formation of benign teratomas in the recipients[43-46]. Those risks are also present in all cultured cells, as demonstrated by Irioda *et al*[47].

As discussed by the aforementioned authors, an ES-derived teratoma is not essentially malignant, but its natural propensity to grow makes it potentially dangerous when implanted into an individual and, as such, a crippling obstacle on the path to ES cell therapeutics[48,49]. Recent experiments suggest that the formation of a teratoma may be dependent upon experimental conditions. For instance, Bjorklund *et al*[50] have shown that teratoma formation could be prevented in a majority of cases, when pre-differentiated mouse ES cells were implanted into the brains of rats at a very low density. Asano *et al*[51] showed that ES cells implanted allogenically into a non-human primate fetus in utero formed a teratoma when developed in a natural cavity, but conversely integrated normally in tissues when implanted within various organs. Therefore, teratoma formation does not appear to be an unavoidable consequence of ES cell implantation but rather as a phenomenon, the mechanisms of which require further investigation in order to identify the safest procedures for clinical application. Tumorigenicity demands the use of an extensively characterised, pure, differentiated cell population as well as rigorous cell screening.

The negative selection of Oct4 (undifferentiated cell marker) expressing cells might be a solution. New strategies and methodologies need to be developed to isolate the terminally differentiated cells. ES cell implants can be tagged with some kind of death signal in such a way that when they start to form tumors, or cause severe complications, they can be cleared from the body, leaving the host unaffected. Other safeguards proposed to purify CMCs, such as flow cytometry, cell sorting using cardiomyocyte-specific fluorescent dye or cardiac plasma membrane surface marker, and other strategies reviewed elsewhere, would further enhance the safety profile of these exogenously derived CMCs. As yet, there is no validated solution to this problem[51-54].

Hence, it is probably unrealistic to assume that an approach designed to improve cardiac differentiation would be applicable to all hESC lines. Clearly, systematic characterisation is necessary in order to identify sub-categories of hESC lines. According to Stojkovic *et al*[55], one possible solution to this problem is the establishment of national or international hESC banks, which would allow comparable and detailed characterisation of deposited cells and provide scientists with all the necessary information to choose the most suitable hESC line for their own research[56].

SOMATIC CELL NUCLEAR TRANSFER

Recently, high-profile reports of the derivation of human embryonic stem cells from human blastocysts produced by somatic cell nuclear transfer (SCNT) have highlighted the possibility of making autologous cell lines specific to individual patients[55]. Given the range of immunophenotypes of hESC lines currently available, rejection of the differentiated cells by the host is a potentially serious problem. SCNT offers a means of circumventing this by producing embryonic stem cells of the same genotype as the donor. However, this technique is not without problems since it requires the resetting of the gene expression programme of a somatic cell to a state consistent with

embryonic development[43,56,57].

The use of SCNT is currently under investigation from several points of view (ethical, scientific, technical/technological) and it has promising potential for the treatment of a variety of degenerative diseases. Furthermore, with the advent of other techniques such as xenofree, and direct differentiation of resident cells to CMCs, this may offer additional and exciting avenues for autologous cell therapy in the future[58-60].

ESC and SCNT have excellent perspectives for future study in preclinical models of cardiomyopathy, such as Chagasic or ischemic, but there are still many questions to be answered and those cells have not yet been evaluated in this preclinical model.

CONCLUSION

The success of stem cell therapy in a preclinical model for treating Chagas Disease is unsuccessful in human translation. Solutions are needed to provide acceptable levels of safety and strict quality control that would make possible the clinical applications of conducting therapy with stem cells in Chagas cardiomyopathy. Addressing the challenges associated with future research may ensure the success of stem cell therapy in the improvement of preclinical models and the treatment of Chagas Disease.

REFERENCES

1 **Perin EC**, Silva GV. Stem cell therapy for cardiac diseases. *Curr Opin Hematol* 2004; **11**: 399-403 [PMID: 15548994 DOI: 10.1097/01.moh.0000143359.77689.aa]

2 **Ribeiro Dos Santos R**, Rassi S, Feitosa G, Grecco OT, Rassi A, da Cunha AB, de Carvalho VB, Guarita-Souza LC, de Oliveira W, Tura BR, Soares MB, Campos de Carvalho AC. Cell therapy in Chagas cardiomyopathy (Chagas arm of the multicenter randomized trial of cell therapy in cardiopathies study): a multicenter randomized trial. *Circulation* 2012; **125**: 2454-2461 [PMID: 22523306 DOI: 10.1161/CIRCULATIONAHA.111.067785]

3 **Mann DL**. Mechanisms and models in heart failure: A combinatorial approach. *Circulation* 1999; **100**: 999-1008 [PMID: 10468532 DOI: 10.1161/CIRCULATIONAHA.104.500546]

4 **Givertz MM**, Colucci WS. New targets for heart-failure therapy: endothelin, inflammatory cytokines, and oxidative stress. *Lancet* 1998; **352** Suppl 1: SI34-SI38 [PMID: 9736478 DOI: 10.1016/S0140-6736(98)90017-4]

5 **Nabors GS**, Tarleton RL. Differential control of IFN-gamma and IL-2 production during Trypanosoma cruzi infection. *J Immunol* 1991; **146**: 3591-3598 [PMID: 1902857]

6 **Carvalho KA**, Guarita-Souza LC, Rebelatto CL, Senegaglia AC, Hansen P, Mendonça JG, Cury CC, Francisco JC, Brofman PR. Could the coculture of skeletal myoblasts and mesenchymal stem cells be a solution for postinfarction myocardial scar? *Transplant Proc* 2004; **36**: 991-992 [PMID: 15194344 DOI: 10.1016/j.transproceed.2004.03.056]

7 **Soares MB**, Pontes-De-Carvalho L, Ribeiro-Dos-Santos R. The pathogenesis of Chagas' disease: when autoimmune and parasite-specific immune responses meet. *An Acad Bras Cienc* 2001; **73**: 547-559 [PMID: 11743602 DOI: 10.1590/S0001-37652001000400008]

8 **Soares MB**, Lima RS, Rocha LL, Takyia CM, Pontes-de-Carvalho L, de Carvalho AC, Ribeiro-dos-Santos R. Transplanted bone marrow cells repair heart tissue and reduce myocarditis in chronic chagasic mice. *Am J Pathol* 2004; **164**: 441-447 [PMID: 14742250 DOI: 10.1016/S0002-9440(10)63134-3]

9 **Pontes-de-Carvalho L**, Santana CC, Soares MB, Oliveira GG, Cunha-Neto E, Ribeiro-dos-Santos R. Experimental chronic Chagas' disease myocarditis is an autoimmune disease preventable by induction of immunological tolerance to myocardial antigens. *J Autoimmun* 2002; **18**: 131-138 [PMID: 11908945 DOI: 10.1006/jaut.2001.0574]

10 **Weissman IL**, Shizuru JA. The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. *Blood* 2008; **112**: 3543-3553 [PMID: 18948588 DOI: 10.1182/blood-2008-08-078220]

11 **Ma T**, Xie M, Laurent T, Ding S. Progress in the reprogramming of somatic cells. *Circ Res* 2013; **112**: 562-574 [PMID: 23371904 DOI: 10.1161/CIRCRESAHA.111.249235]

12 **Abdelwahid E**, Siminiak T, Guarita-Souza LC, Teixeira de Carvalho KA, Gallo P, Shim W, Condorelli G. Stem cell therapy in heart diseases: a review of selected new perspectives, practical considerations and clinical applications. *Curr Cardiol Rev* 2011; **7**: 201-212 [PMID: 22758618 DOI: 10.2174/157340311798220502]

13 **Rossi MA**, Gonçalves S, Ribeiro-dos-Santos R. Experimental Trypanosoma cruzi cardiomyopathy in BALB/c mice. The potential role of intravascular platelet aggregation in its genesis. *Am J Pathol* 1984; **114**: 209-216 [PMID: 6230012]

14 **Rocha A**, de Meneses AC, da Silva AM, Ferreira MS, Nishioka SA, Burgarelli MK, Almeida E, Turcato Júnior G, Metze K, Lopes ER. Pathology of patients with Chagas' disease and acquired immunodeficiency syndrome. *Am J Trop Med Hyg* 1994; **50**: 261-268 [PMID: 8147485]

15 **Jasmin LA**, Koba W, Tanowitz HB, Mendez-Otero R, Campos de Carvalho AC, Spray DC. Mesenchymal bone marrow cell therapy in a mouse model of chagas disease. Where do the cells go? *PLoS Negl Trop Dis* 2012; **6**: e1971 [PMID: 23272265 DOI: 10.1371/journal.pntd.0001971]

16 **Carvalho KA**, Guarita-Souza LC, Hansen P, Rebelatto CL, Senegaglia AC, Miyague N, Olandoski M, Francisco JC, Furuta M, Gremski W. Cell transplantation after the coculture of skeletal myoblasts and mesenchymal stem cells in the regeneration of the myocardium scar: an experimental study in rats. *Transplant Proc* 2006; **38**: 1596-1602 [PMID: 16797364 DOI: 10.1016/j.transproceed.2006.03.023]

17 **Guarita-Souza LC**, Carvalho KA, Woitowicz V, Rebelatto C, Senegaglia A, Hansen P, Miyague N, Francisco JC, Olandoski M, Faria-Neto JR, Brofman P. Simultaneous autologous transplantation of cocultured mesenchymal stem cells and skeletal myoblasts improves ventricular function in a murine model of Chagas disease. *Circulation* 2006; **114**: I120-I124 [PMID: 16820560 DOI: 10.1161/CIRCULATIONAHA.105.000646]

18 **Sabino EC**, Ribeiro AL, Salemi VM, Di Lorenzo Oliveira C, Antunes AP, Menezes MM, Ianni BM, Nastari L, Fernandes F, Patavino GM, Sachdev V, Capuani L, de Almeida-Neto C, Carrick DM, Wright D, Kavounis K, Goncalez TT, Carneiro-Proietti AB, Custer B, Busch MP, Murphy EL. Ten-year incidence of Chagas cardiomyopathy among asymptomatic Trypanosoma cruzi-seropositive former blood donors. *Circulation* 2013; **127**: 1105-1115 [PMID: 23393012 DOI: 10.1161/CIRCULATIONAHA.112.123612]

19 **Hagège AA**, Marolleau JP, Vilquin JT, Alhéritière A, Peyrard S, Duboc D, Abergel E, Messas E, Mousseaux E, Schwartz K, Desnos M, Menasché P. Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. *Circulation* 2006; **114**: I108-I113 [PMID: 16820558 DOI: 10.1161/CIRCULATIONAHA.105.000521]

20 **Mummery C**, Ward D, van den Brink CE, Bird SD, Doevendans PA, Opthof T, Brutel de la Riviere A, Tertoolen L, van der Heyden M, Pera M. Cardiomyocyte differentiation of mouse and human embryonic stem cells. *J*

Anat 2002; **200**: 233-242 [PMID: 12033727 DOI: 10.1046/j.1469-7580.2002.00031.x]
21 **Kehat I**, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 2001; **108**: 407-414 [PMID: 11489934 DOI: 10.1172/JCI12131]
22 **Kehat I**, Khimovich L, Caspi O, Gepstein A, Shofti R, Arbel G, Huber I, Satin J, Itskovitz-Eldor J, Gepstein L. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol* 2004; **22**: 1282-1289 [PMID: 15448703 DOI: 10.1038/nbt1014]
23 **Menasché P**. Embryonic stem cells pace the heart. *Nat Biotechnol* 2004; **22**: 1237-1238 [PMID: 15470458 DOI: 10.1038/nbt1004-1237]
24 **Odorico JS**, Kaufman DS, Thomson JA. Multilineage differentiation from human embryonic stem cell lines. *Stem Cells* 2001; **19**: 193-204 [PMID: 11359944 DOI: 10.1634/stemcells.19-3-1]
25 **Carpenter MK**, Rosler ES, Fisk GJ, Brandenberger R, Ares X, Miura T, Lucero M, Rao MS. Properties of four human embryonic stem cell lines maintained in a feeder-free culture system. *Dev Dyn* 2004; **229**: 243-258 [PMID: 14745950 DOI: 10.1002/dvdy.10431]
26 **Rosler ES**, Fisk GJ, Ares X, Irving J, Miura T, Rao MS, Carpenter MK. Long-term culture of human embryonic stem cells in feeder-free conditions. *Dev Dyn* 2004; **229**: 259-274 [PMID: 14745951 DOI: 10.1002/dvdy.10430]
27 **Abeyta MJ**, Clark AT, Rodriguez RT, Bodnar MS, Pera RA, Firpo MT. Unique gene expression signatures of independently-derived human embryonic stem cell lines. *Hum Mol Genet* 2004; **13**: 601-608 [PMID: 14749348 DOI: 10.1093/hmg/ddh0]
28 **Richards M**, Fong CY, Chan WK, Wong PC, Bongso A. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. *Nat Biotechnol* 2002; **20**: 933-936 [PMID: 12161760 DOI: 10.1038/nbt726]
29 **Pekkanen-Mattila M**, Kerkelä E, Tanskanen JM, Pietilä M, Pelto-Huikko M, Hyttinen J, Skottman H, Suuronen R, Aalto-Setälä K. Substantial variation in the cardiac differentiation of human embryonic stem cell lines derived and propagated under the same conditions--a comparison of multiple cell lines. *Ann Med* 2009; **41**: 360-370 [PMID: 19165643 DOI: 10.1080/07853890802609542]
30 **Heng BC**, Haider HK, Sim EK, Cao T, Tong GQ, Ng SC. Comments about possible use of human embryonic stem cell-derived cardiomyocytes to direct autologous adult stem cells into the cardiomyogenic lineage. *Acta Cardiol* 2005; **60**: 7-12 [PMID: 15779844 DOI: 10.2143/AC.60.1.2005041]
31 **Heng BC**, Cao T, Haider HK, Wang DZ, Sim EK, Ng SC. An overview and synopsis of techniques for directing stem cell differentiation in vitro. *Cell Tissue Res* 2004; **315**: 291-303 [PMID: 14758536 DOI: 10.1007/s00441-003-0847-5]
32 **Heng BC**, Cao T, Tong GQ, Ng SC. Potential utility of cell-permeable transcription factors to direct stem cell differentiation. *Stem Cells Dev* 2004; **13**: 460-462 [PMID: 15588503 DOI: 10.1089/scd.2004.13.460]
33 **Heng BC**, Haider HKh, Sim EK, Cao T, Ng SC. Strategies for directing the differentiation of stem cells into the cardiomyogenic lineage in vitro. *Cardiovasc Res* 2004; **62**: 34-42 [PMID: 15023550 DOI: 10.1016/j.cardiores.2003.12.022]
34 **Stevens KR**, Kreutziger KL, Dupras SK, Korte FS, Regnier M, Muskheli V, Nourse MB, Bendixen K, Reinecke H, Murry CE. Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. *Proc Natl Acad Sci U S A* 2009; **106**: 16568-16573 [PMID: 19805339 DOI: 10.1073/pnas.0908381106]
35 **Martin MJ**, Muotri A, Gage F, Varki A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. *Nat Med* 2005; **11**: 228-232 [PMID: 15685172 DOI: 10.1038/nm1181]
36 **Ellerström C**, Strehl R, Moya K, Andersson K, Bergh C, Lundin K, Hyllner J, Semb H. Derivation of a xeno-free human embryonic stem cell line. *Stem Cells* 2006; **24**: 2170-2176 [PMID: 16741223 DOI: 10.1634/stemcells.2006-0130]
37 **Rajala K**, Hakala H, Panula S, Aivio S, Pihlajamäki H, Suuronen R, Hovatta O, Skottman H. Testing of nine different xeno-free culture media for human embryonic stem cell cultures. *Hum Reprod* 2007; **22**: 1231-1238 [PMID: 17251355 DOI: 10.1093/humrep/del523]
38 **Amit M**, Margulets V, Segev H, Shariki K, Laevsky I, Coleman R, Itskovitz-Eldor J. Human feeder layers for human embryonic stem cells. *Biol Reprod* 2003; **68**: 2150-2156 [PMID: 12606388 DOI: 10.1095/biolreprod.102.012583]
39 **Unger C**, Skottman H, Blomberg P, Dilber MS, Hovatta O. Good manufacturing practice and clinical-grade human embryonic stem cell lines. *Hum Mol Genet* 2008; **17**: R48-R53 [PMID: 18632697 DOI: 10.1093/hmg/ddn079]
40 **Pekkanen-Mattila M**, Chapman H, Kerkelä E, Suuronen R, Skottman H, Koivisto AP, Aalto-Setälä K. Human embryonic stem cell-derived cardiomyocytes: demonstration of a portion of cardiac cells with fairly mature electrical phenotype. *Exp Biol Med* (Maywood) 2010; **235**: 522-530 [PMID: 20407085 DOI: 10.1258/ebm.2010.009345]
41 **Drukker M**, Katz G, Urbach A, Schuldiner M, Markel G, Itskovitz-Eldor J, Reubinoff B, Mandelboim O, Benvenisty N. Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc Natl Acad Sci USA* 2002; **99**: 9864-9869 [PMID: 12114532 DOI: 10.1073/pnas.142298299]
42 **Robertson NJ**, Brook FA, Gardner RL, Cobbold SP, Waldmann H, Fairchild PJ. Embryonic stem cell-derived tissues are immunogenic but their inherent immune privilege promotes the induction of tolerance. *Proc Natl Acad Sci USA* 2007; **104**: 20920-20925 [PMID: 18093946 DOI: 10.1073/pnas.0710265105]
43 **Hwang WS**, Ryu YJ, Park JH, Park ES, Lee EG, Koo JM, Jeon HY, Lee BC, Kang SK, Kim SJ, Ahn C, Hwang JH, Park KY, Cibelli JB, Moon SY. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science* 2004; **303**: 1669-1674 [PMID: 14963337 DOI: 10.1126/science.1094515]
44 **Armstrong L**, Lako M, Dean W, Stojkovic M. Epigenetic modification is central to genome reprogramming in somatic cell nuclear transfer. *Stem Cells* 2006; **24**: 805-814 [PMID: 16282443 DOI: 10.1634/stemcells.2005-0350]
45 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147 [PMID: 9804556 DOI: 10.1126/science.282.5391.1145]
46 **Thomson JA**, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP. Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci USA* 1995; **92**: 7844-7848 [PMID: 7544005 DOI: 10.1073/pnas.92.17.7844]
47 **Irioda AC**, Zocche L, Souza CMCO, Ferreira RJ, Aliprandini E, CunhaRC, Francisco JC, Guarita-Souza LC, Malvezzi M, Beltrame MP, Mesquita LAF, Kuczera D, Chachques JC, Carvalho KAT. Pap test as the first step in screening genetic stability in cell-based therapy. *J Stem Cell Res Ther* 2011; **1**: 101-106 [DOI: 10.4172/2157-7633.1000106]
48 **Doss MX**, Koehler CI, Gissel C, Hescheler J, Sachinidis A. Embryonic stem cells: a promising tool for cell replacement therapy. *J Cell Mol Med* 2004; **8**: 465-473 [PMID: 15601575 DOI: 10.1111/j.1582-4934.2004.tb00471.x]
49 **Mitjavila-Garcia MT**, Simonin C, Peschanski M. Embryonic stem cells: meeting the needs for cell therapy. *Adv Drug Deliv Rev* 2005; **57**: 1935-1943 [PMID: 16257083 DOI: 10.1016/j.addr.2005.06.001]
50 **Bjorklund LM**, Sánchez-Pernaute R, Chung S, Andersson T,

Chen IY, McNaught KS, Brownell AL, Jenkins BG, Wahlestedt C, Kim KS, Isacson O. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci USA* 2002; **99**: 2344-2349 [PMID: 11782534 DOI: 10.1073/pnas.022438099]

51 **Asano T**, Ageyama N, Takeuchi K, Momoeda M, Kitano Y, Sasaki K, Ueda Y, Suzuki Y, Kondo Y, Torii R, Hasegawa M, Ookawara S, Harii K, Terao K, Ozawa K, Hanazono Y. Engraftment and tumor formation after allogeneic in utero transplantation of primate embryonic stem cells. *Transplantation* 2003; **76**: 1061-1067 [PMID: 14557753 DOI: 10.1097/01.TP.0000090342.85649.81]

52 **Hattori F**, Chen H, Yamashita H, Tohyama S, Satoh YS, Yuasa S, Li W, Yamakawa H, Tanaka T, Onitsuka T, Shimoji K, Ohno Y, Egashira T, Kaneda R, Murata M, Hidaka K, Morisaki T, Sasaki E, Suzuki T, Sano M, Makino S, Oikawa S, Fukuda K. Nongenetic method for purifying stem cell-derived cardiomyocytes. *Nat Methods* 2010; **7**: 61-66 [PMID: 19946277 DOI: 10.1038/nmeth.1403]

53 **Van Hoof D**, Dormeyer W, Braam SR, Passier R, Monshouwer-Kloots J, Ward-van Oostwaard D, Heck AJ, Krijgsveld J, Mummery CL. Identification of cell surface proteins for antibody-based selection of human embryonic stem cell-derived cardiomyocytes. *J Proteome Res* 2010; **9**: 1610-1618 [PMID: 20088484 DOI: 10.1021/pr901138a]

54 **Hattori F**, Fukuda K. Strategies for ensuring that regenerative cardiomyocytes function properly and in cooperation with the host myocardium. *Exp Mol Med* 2010; **42**: 155-165 [PMID: 20164677 DOI: 10.1016/j.trre.2011.09.003]

55 **Stojkovic M**, Lako M, Strachan T, Murdoch A. Derivation, growth and applications of human embryonic stem cells. *Reproduction* 2004; **128**: 259-267 [PMID: 15333777 DOI: 10.1530/rep.1.00243]

56 **Reubinoff BE**, Itsykson P, Turetsky T, Pera MF, Reinhartz E, Itzik A, Ben-Hur T. Neural progenitors from human embryonic stem cells. *Nat Biotechnol* 2001; **19**: 1134-1140 [PMID: 11731782 DOI: 10.1038/nbt1201-1134]

57 **Lanzendorf SE**, Boyd CA, Wright DL, Muasher S, Oehninger S, Hodgen GD. Use of human gametes obtained from anonymous donors for the production of human embryonic stem cell lines. *Fertil Steril* 2001; **76**: 132-137 [PMID: 11438331 DOI: 10.1016/S0015-0282(01)01825-8]

58 **Rodríguez-Pizà I**, Richaud-Patin Y, Vassena R, González F, Barrero MJ, Veiga A, Raya A, Izpisúa Belmonte JC. Reprogramming of human fibroblasts to induced pluripotent stem cells under xeno-free conditions. *Stem Cells* 2010; **28**: 36-44 [PMID: 19890879 DOI: 10.1002/stem.248]

59 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]

60 **Ieda M**, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 2010; **142**: 375-386 [PMID: 20691899 DOI: 10.1016/j.cell.2010.07.002]

P- Reviewer: Villalta F **S- Editor**: Ma YJ
L- Editor: A **E- Editor:** Yan JL

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5500/wjt.v3.i4.127

World J Transplant 2013 December 24; 3(4): 127-133
ISSN 2220-3230 (online)

BRIEF ARTICLE

Isolated small bowel transplantation outcomes and the impact of immunosuppressants: Experience of a single transplant center

Ibtesam A Hilmi, Raymond M Planinsic, Ramona Nicolau-Raducu, Daniela Damian, Ali Al-Khafaji, Tetsuro Sakai, Kareem Abu-Elmagd

Ibtesam A Hilmi, Raymond M Planinsic, Daniela Damian, Tetsuro Sakai, Department of Anesthesiology, University of Pittsburgh Medical Center, Presbyterian Hospital, Pittsburgh, PA 15213, United States
Ramona Nicolau-Raducu, Department of Anesthesiology, Ochsner Health System, New Orleans, LA 70121, United States
Ali Al-Khafaji, Transplant Intensive Care Unit, Department of Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, United States
Kareem Abu-Elmagd, Center for Gut Rehabilitation and Transplantation, Cleveland, OH 44195, United States
Author contributions: Hilmi IA wrote the study protocol and study design, collected the data, and wrote the manuscript; Planinsic RM reviewed the scientific material and edited the manuscript; Nicolau-Raducu R and Damian D collected the data and performed the statistical analysis; Al-Khafaji A reviewed the scientific material and study design and participated in data collection and analysis; Sakai T and Abu-Elmagd K provided the data, served as scientific advisors, and participated in the manuscript writing.
Supported by The Department of Anesthesiology at the University of Pittsburgh Medical Center
Correspondence to: Ibtesam A Hilmi, MBCHB, FRCA, Associate Professor of Anesthesiology, Department of Anesthesiology, University of Pittsburgh Medical Center, Presbyterian Hospital, C-wing Suite 200,200 Lothrop St., Pittsburgh, PA 15213, United States. hilmiia@anes.upmc.edu
Telephone: +1-412-6473262 Fax: +1-412-6476290
Received: March 15, 2013 Revised: June 4, 2013
Accepted: July 18, 2013
Published online: December 24, 2013

Abstract

AIM: To investigate patient and graft outcomes in isolated small bowel transplant (SBTx) recipients and immunosuppressant induction agent impact on outcomes.

METHODS: A retrospective review of the perioperative data of patients who underwent SBTx transplant during an 8-year period was conducted. The intraoperative data were: patient demographics, etiology of short gut syndrome, hemodynamic parameters, coagulation profiles, intraoperative fluid and blood products transfused, and development of post-reperfusion. The postoperative data were: hospital/intensive care unit stays, duration of mechanical ventilation, postoperative incidence of acute kidney injury, and 1-year patient and graft outcomes. The effects of the three immunosuppressant induction agents (Zenapax, Thymoglobulin, Campath) on patient and graft outcomes were reviewed.

RESULTS: During the 8-year period there were 77 patients; 1-year patient and graft survival were 95% and 86% respectively. Sixteen patients received Zenapax, 22 received Thymoglobulin, and 39 received Campath without effects on patient or graft survival (P = 0.90, P = 0.14, respectively). The use of different immune induction agents did not affect the incidence of rejection and infection during the first 90 postoperative days (P = 0.072, P = 0.29, respectively). The Zenapax group received more intraoperative fluid and blood products and were coagulopathic at the end of surgery. Zenapax and Thymoglobulin significantly increased serum creatinine at 48 h (P = 0.023) and 1 wk (P = 0.001) post-transplant, but none developed renal failure or required dialysis at the end of the first year.

CONCLUSION: One-year patient and graft survival were 95% and 86%, respectively. The use of different immunosuppressant induction agents may affect the intraoperative course and short-term postoperative morbidities, but not 1-year patient and graft outcomes.

Key words: Small bowel transplant; Outcomes; Immune induction agents; Zenapax; Thymoglobulin; Campath

Core tip: Small bowel transplant (SBTx) is the treatment of choice for patients with intestinal failure. However, patient and graft survival can be affected by multiple factors, such as the choice of immunosuppressant and immune induction agent. Studying the effects of these agents may help care providers customize the immunosuppressant protocol to the individual patient. In this study, we reviewed in great detail how different immune induction agents can impact the intraoperative and postoperative course, as well as the short term outcome of these patients. Such information can be of great value to physicians who treat SBTx recipients.

Hilmi IA, Planinsic RM, Nicolau-Raducu R, Damian D, Al-Khafaji A, Sakai T, Abu-Elmagd K. Isolated small bowel transplantation outcomes and the impact of immunosuppressants: Experience of a single transplant center. *World J Transplant* 2013; 3(4): 127-133 Available from: URL: http://www.wjgnet.com/2220-3230/full/v3/i4/127.htm DOI: http://dx.doi.org/10.5500/wjt.v3.i4.127

INTRODUCTION

Isolated small bowel transplant (SBTx) is a quickly-growing curative procedure for patients with short gut syndrome (SGS)[1]. Improvements in surgical techniques, immunosuppressant drugs, and anesthetic management[2] have resulted in great improvement of patient outcome. In this retrospective single-center study, we reviewed the medical records of 77 consecutive patients who underwent first time isolated SBTx. Patient and graft outcome, along with the effects of different immunosuppressant induction agents, are reported.

MATERIALS AND METHODS

After Institutional Review Board approval, the medical records of 77 consecutive adult patients who underwent first time isolated SBTx during an 8-year period (April 2000-June 2007) were reviewed. Preoperative data included patient demographics, etiology of SGS, and renal function status. Intraoperative hemodynamic data were recorded at six time points during the surgery: I (baseline before the surgical incision), I +60 (60 min after the surgical incision), II (completion of enterectomy/dissection), III+5 (5 min post-reperfusion), III+60 (60 min post-reperfusion), and IV (at completion of surgery). These data were: heart rate, mean arterial blood pressure (MABP), central venous pressure, pulmonary artery wedge pressure, mean pulmonary artery pressure, cardiac output (CO), systemic and pulmonary vascular resistance (SVR, PVR), end-diastolic volume, right ventricular ejection fraction, and mixed venous oxygen saturation. At each of these six time points, a thromboelastogram (TEG) was performed and the results were recorded and analyzed.

In order to investigate the severity of the post reperfusion syndrome (PRS) in SBTx, patients were divided into two groups according to PRS severity: patients with mild PRS and patients with significant PRS[3]. PRS was defined as mild when the decrease in blood pressure and/or HR was < 30% baseline, was short-lived (≤ 5 min), and responded to the administration of small doses of vasopressors [calcium chloride (1 g IV) and/or epinephrine (≤ 100 μg) IV] without requiring continuous infusion of these vasopressors during the remaining transplantation procedure. PRS was defined as significant when severe hemodynamic instability occurred, such as persistent hypotension (> 30% baseline), asystole or hemodynamically significant arrhythmias requiring intraoperative infusion of vasopressors, and fibrinolysis that required treatment with antifibrinolytic agents.

Patients received one of three immunosuppressant induction agents: Zenapax, Campath, or Thymoglobulin; the effects of these agents on patient and graft outcomes were reviewed. As part of the immunosuppressant regimen, all patients received 1 g methylprednisolone before reperfusion of the graft and postoperative tacrolimus.

Postoperative data collected were: hospital/intensive care unit (ICU) stay, days requiring postoperative mechanical ventilation, postoperative incidence of acute kidney injury (AKI), incidence of infection and rejection within the first 90 postoperative days, and 1-year patient and graft outcomes. To document the presence of post-transplant infection, we utilized the International Sepsis Forum Consensus Conference definition of infection in the ICU[4]. AKI was defined by modified RIFLE (risk, injury, failure, loss, end-stage renal disease) criteria as recommended by the Kidney Disease Improving Global Outcomes (KDIGO) AKI guideline, but without urine output data. According to the KDIGO, AKI was defined as a 50% increase in SCr from the baseline (preoperative value as in our study) or a 0.3 mg/dL increase within 48 h[5-7].

The statistical analysis was performed using SPSS Statistics 17.0 software (SSPS Inc., Chicago, IL). Descriptive statistics were reported as mean ± SD. For categorical data, the χ^2 test or Fisher exact test were used and for normally distributed continuous variable data, the paired *t*-test or ANOVA were used. For analysis of the continuous variables that were not normally distributed, the Kruskal Wallis test was used and the median and range were reported. For patient and graft 1-year survival, Kaplan-Meier survival analysis was used.

RESULTS

Seventy-seven patients received an isolated SBTx during the 8-year study period. Sixteen patients received Zenapax, 22 received Thymoglobulin, and 39 received Campath. Patient demographics were: age (range 28-66 years old, mean of 40), more females than males (26/51) with $P = 0.02$. Etiologies of SGS were: volvulus in 11 patients, vascular in 24, inflammatory in 20, trauma and adhesion in 10, radiation in four, and miscellaneous in eight. Intra-

Table 1 Intraoperative data (mean ± SD, *n* = 77)

	Zenapax (*n* = 16)	Thymoglobulin (*n* = 22)	Campath (*n* = 39)	*P* value
Surgical time (h)	14.16 ± 2.59	12.01 ± 1.73	10.59 ± 1.59	< 0.000
Cold ischemia time (min)	514.0 ± 86.73	480.18 ± 111.60	441.0 ± 100.33	0.05
Warm ischemia time (min)	31.18 ± 5.19	30.95 ± 4.94	32.18 ± 4.62	0.636
Crystalloids (mL)	7214.67 ± 2286.39	5622.73 ± 1707.95	4705.26 ± 1791.26	0.0002
Colloids (mL)	6383.33 ± 2277.34	4428.64 ± 1611.94	3721.05 ± 1516.51	< 0.0001
Packed red cell (units)	6.47 ± 3.44	3.45 ± 1.95	3.66 ± 2.39	0.0008
Fresh frozen plasma (units)	0.73 ± 1.71	0.36 ± 1.05	1 ± 1.61	0.208
Cryoprecipitate (units)	0	0.27 ± 1.27	1.58 ± 3.32	0.377
Platelets (units)	1.20 ± 3.36	4.36 ± 8.52	3.95 ± 6.58	0.541

Table 2 Intraoperative changes in the thromboelastogram tracing (mean ± SD)

	Zenapax (*n* = 16)	Thymoglobulin (*n* = 22)	Campath (*n* = 39)	*P* value
Stage-Ⅰ (baseline)				
R Ⅰ	9.26 ± 3.6	9.09 ± 3.88	7.36 ± 4.35	0.195
K Ⅰ	2.72 ± 0.91	2.73 ± 0.97	2.21 ± 2.96	0.630
ANG Ⅰ	71.64 ± 5.46	65.9 ± 14.1	66.1 ± 12.25	0.291
MA Ⅰ	65.11 ± 11.1	59.53 ± 14.56	66.04 ± 13.72	0.223
Stage-Ⅱ (organ on the field)				
R Ⅱ	9.12 ± 5.23	7.36 ± 3.17	7.15 ± 3.54	0.327
K Ⅱ	4.26 ± 1.53	3.99 ± 2.1	3.10 ± 2.12	0.176
ANG Ⅱ	64.25 ± 8.26	60.82 ± 11.66	55 ± 15.72	0.109
MA Ⅱ	53.58 ± 10.42	53.71 ± 9.7	54.44 ± 15.16	0.974
Stage-Ⅲ (reperfusion)				
R Ⅲ	9.3 ± 2.7	7.44 ± 2.53	7.74 ± 3.4	0.356
K Ⅲ	6.13 ± 2.56	4.77 ± 2.1	3.9 ± 2.54	0.088
ANG Ⅲ	56.13 ± 9.23	58.6 ± 7.36	49.2 ± 15.57	0.072
MA Ⅲ	47.56 ± 12.5	50.7 ± 10.67	48.67 ± 16.1	0.855
Closing the abdominal incision (final)				
R end	9.26 ± 2.58	8.77 ± 3.39	6.38 ± 2.31	0.011[a]
K end	6.21 ± 2.83	5.13 ± 2.1	2.79 ± 1.62	< 0.0001[a]
ANG end	56.75 ± 12.8	57.47 ± 7.6	58.1 ± 11.13	0.949
MA end	50.56 ± 8.51	51.32 ± 11.76	55.46 ± 9.23	0.339

[a]*P* < 0.05. R: R-time (min); K: Tangential line in the thromboelastogram tracing (min); ANG: α-angle (degree); MA: Maximum amplitude (millimeter).

operative data showed that patients who received Zenapax had significantly longer surgical times ($P = 0.0001$), longer cold ischemia times ($P = 0.05$), and required more crystalloid ($P = 0.002$), more colloid ($P = 0.00001$), and packed red cells ($P = 0.0008$) (Table 1). The intraoperative coagulation profile (Table 2) as monitored by TEG showed no significant differences between the groups until the completion of surgery. At that point, the Zenapax group had a longer R-time ($P = 0.011$) and K-time ($P = 0.0001$), indicating less coagulability. Significant changes were found in almost all hemodynamic parameters during the reperfusion phase when compared to the baseline readings (Table 3). These changes were reflected in a drop in the SVR ($P = 0.0001$), increase in CO ($P = 0.0001$), and decrease in MABP ($P = 0.0001$); however, these changes in hemodynamic parameters were considered as mild PRS according to our definition. There was a trend toward more hemodynamic instability in the Zenapax group, but it did not reach statistical significance ($P < 0.065$).

Changes in postoperative SCr and the effects of the different immune induction agents showed a significant increase in SCr in patients who received Zenapax ($P = 0.023$) and Thymoglobulin ($P = 0.001$). Overall, 15 patients developed AKI (19.5%) during the first 72 h post-transplant, which increased to 31.2% (24 patients) after completion of the first postoperative week, but none progressed to renal failure or required dialysis at any time during the first post-transplant year. Classical outcome criteria showed a mean ICU stay of 5 d (range 4-62 d), mean hospital stay of 26 d (range 7-89 d), and mean time on ventilator of 2 d (range 1-95 d). Infection within the first 90 postoperative days was reported in 24 patients with no prevalence among immune induction agent used ($P = 0.29$).

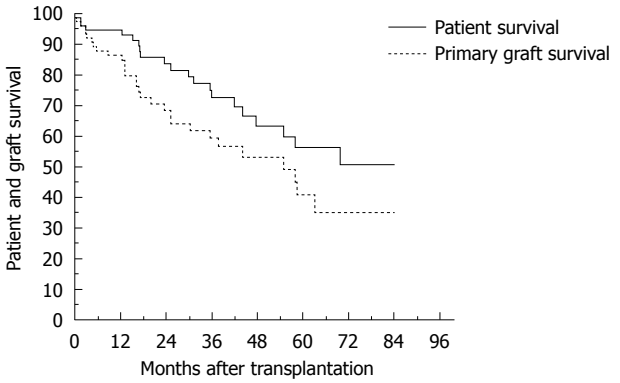

Figure 1 Overall patient and graft survival in months.

Forty-three rejection episodes occurred during the first 90 d post-transplant; 21 were considered severe episodes. The use of different immunosuppressant induction agents had no impact on the incidence of rejection within the first 90 d post-transplant. The 1-year patient survival was 95% and the 1-year graft survival was 86% (Figure 1), with no impact of immunosuppressant induction agent type on graft and patient survival (Figure 2, $P = 0.09$, $P = 0.14$, respectively).

DISCUSSION

Patients with SGS presenting for isolated SBTx are known to have multiple problems related to the absence of intestinal functions and/or chronic dependency on total parenteral nutrition (TPN). One of the challenging issues

Table 3 Intraoperative hemodynamic data at baseline and reperfusion (mean ± SD)

	Baseline	Reperfusion	*P* value
Cardiac output (L/min)	7.32 ± 1.88	9.1 ± 2.62	< 0.0001
Ejection fraction (%)	38.14 ± 7.78	39.75 ± 7.76	0.183
End diastolic volume (mL)	216.24 ± 55.82	242.34 ± 64.54	< 0.0001
Mixed venous oxygen saturation	83.80 ± 3.99	85.17 ± 4.37	0.017
Heart rate (beat/min)	89.81 ± 13.49	101.41 ± 13.71	< 0.0001
Mean arterial blood pressure	77.71 ± 9.47	71.29 ± 11.64	< 0.0001
Systemic vascular resistance	793.76 ± 244.67	581.92 ± 196.8	< 0.0001
Mean pulmonary artery pressure	17.52 ± 3.82	20.17 ± 4.8	< 0.0001
Pulmonary vascular resistance	58.07 ± 26.32	52.13 ± 24.76	0.078
Pulmonary capillary wedge pressure	12.42 ± 3.44	14.82 ± 3.8	< 0.0001
Central venous pressure	9.53 ± 3.63	11.42 ± 3.46	< 0.0001

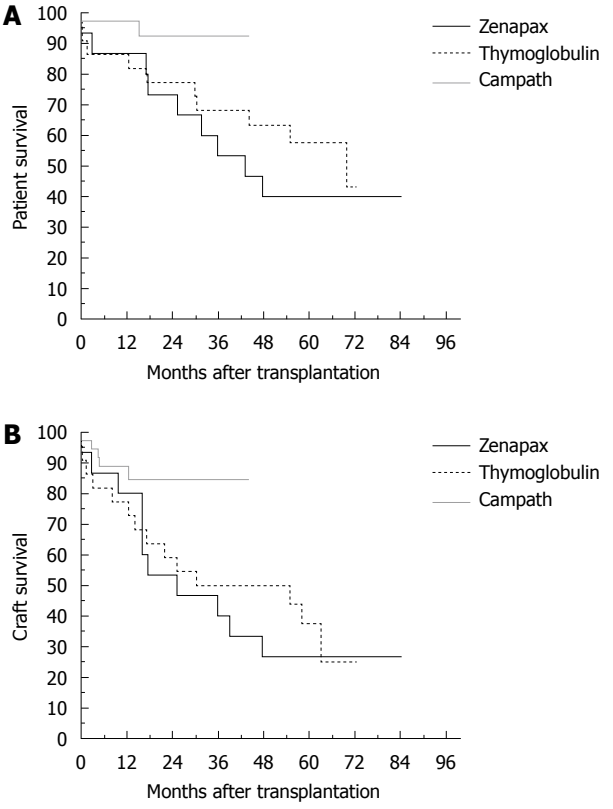

Figure 2 Patient survival (A) and graft survival (B) in months by immunosuppresant induction agent. A: *P* = 0.14; B: *P* = 0.09.

in caring for this group of patients is vanishing vein syndrome due to the use of TPN and utilization of most if not of all of the patient's central venous access[8,9].

Other challenges are attributed to the fact that intestinal grafts are very susceptible to rejection due to high concentration of lymphoid tissue, which may contribute to high incidence of graft *versus* host disease (GVHD)[10-12]. A high level of immunosuppression is required to prevent rejection, which can lead to serious and life threatening sepsis[13]. Selective bowel decontamination and low-dose radiation were implemented in the donor to ameliorate or prevent the occurrence of GVHD reaction. However, in our group of SBTx recipients, we did not see GVHD or lymphoproliferative diseases within the first post-transplant year. The Epstein-Barr virus (EBV) has been linked to the development of lymphoproliferative disorders in SBTx recipients[14]. The absence of post-transplant lymphoproliferative disorders in our series is due to the fact that we reviewed 1 year post-transplant outcomes; also, all the donors in our series were screened for EBV and all recipients were put on EBV prophylaxis regardless of their preoperative viral status. Historically, GVHD affects about 5% of SBTx recipients, which is much higher than in other solid organ transplant recipients[11]. The absence of GVHD in our series may be related to the use of immunosuppressant induction agents. The diagnosis of GVHD was suspected on the basis of clinical symptoms, according to the Consensus Conference on Acute GVHD grading, with its usual attacks on the skin, liver, and gastrointestinal tract[15].

When compared to liver transplant recipients, SBTx recipients have a theoretical advantage in that the graft can be removed in cases of graft-related problems without serious impact on the recipient's well-being. Our results showed improved patient 1-year survival (95%) when compared to the series from England and Wales[16], which was reported to be 57% for cases performed during the late nineties to early 2000s. Our results are better, as they reflect the experience of the surgical team and/or improvement in the immunosuppression protocol and patient selection. The England and Wales study reported a 1-year graft survival of at best, 40%; in our series it was 85%. In our study, the SBTx patients and grafts showed much improvement, even when compared to the first report from our center in 1997[17], which documented patient and graft survival as 73% and 60%, respectively. In most SBTxs that were performed during the nineties, the main immunosuppressants used were tacrolimus and steroids. Tacrolimus is still the main immunosuppressant agent used for SBTx recipients, but new immunoinduction agents have been added while steroids and/or OKT3 have been reserved to treat rejection episodes.

The use of three different immunoinduction agents for this group of patients reflects three periods in the 8-year timeline in which the immunosuppressant protocol at our institution was modified. The overall improvement in SBTx outcome after the introduction of the immu-

noinduction agent Zenapax was documented in children in an early report by the University of Miami[18]. In our report, the use of different induction agents did not affect short-term patient or graft outcomes. However, in the University of Miami report, patient and graft outcomes were better for isolated SBTx than for transplant of combined liver-small bowel or multivisceral grafts. Although this contradicted our report that was published in 2001[1], the difference in the results may be due to the difference in the recipients' age (pediatric *vs* adult). The indications for SBTx at our center are not different from what are used in other transplant centers due to the fact that morbidity and mortality for patients with SGS on TPN are low, especially with advances in technology and pharmacological applications of TPN[19,20]. SBTx is reserved for patients with SGS who develop TPN-related complications, such as loss of venous access, repeated line infection, dehydration, and hepatobilliary complications (cholestasis, fatty liver)[8,19].

Rejection remains the most serious challenge in post-transplant patients. The fact that no single laboratory test can predict or detect rejection makes the diagnosis of rejection even more difficult and an almost impossible task. The diagnosis of rejection depends on adapting a high suspicious index in interpretation of clinical presentation and performing an early endoscopic examination with biopsy. The SBTx service at our center has adopted a standardized protocol for detection and follow up of rejection in SBTx recipients, which includes regular endoscopic examination and biopsy. Treatment of rejection is usually accomplished with OKT3 and supplemental dose of tacrolimus with or without high dose of steroids[21-23].

Intestinal transplantation can be a quite lengthy procedure due to the fact that SBTx recipients typically undergo multiple abdominal surgeries that make the dissection phase very prolonged and complicated. Although the SBTx procedure is longer than the orthotopic liver transplant (OLT) procedure[3], it is associated with less blood loss than OLT (as compared to results from our center); this may be related to the fact that SBTx recipients are hypercoagulable and less likely to lose excessive blood[24].

The presentation of PRS in SBTx recipients was determined and compared to the incidence of PRS in liver transplant recipients, which is a well-documented phenomenon. In this study, criteria established by Hilmi *et al*[3] to define PRS and its severity were used. In the Hilmi study, PRS was found to occur in all liver transplant recipients, with 50% developing mild PRS and the other 50% developing more severe PRS, while in this study, most SBTx recipients suffered mild PRS and almost none suffered severe PRS. Although PRS was common in SBTx recipients, it was not as dramatic as in liver transplant recipients. Residual hypotension, which usually follows initial hypotension in PRS, typically dissipates during the first hour after reperfusion in SBTx.

The duration of ICU and hospital stay can be prolonged; in our series the mean was 3 and 26 d respectively, which is comparable to what is published and known about this procedure. The occurrence of complications, especially infection and rejection, and AKI can further prolong hospital and ICU stay[25]. Tacrolimus-based maintenance therapy is used in most if not all transplant centers world-wide, while steroid and OKT3 are reserved for treatment of rejection episodes. During the last 10 years, application of immune induction agents in SBTx recipients increased such medications include anti-lymphocyte globulin (Thymoglobulin), anti-interleukin receptor globulin (Zenapax), and the latest therapy, alemtuzumab (Campath). The introduction of these agents in clinical practice has significantly reduced the incidence of early rejection and almost eliminated early graft loss as we demonstrated in this study. However, the uses of the immune induction agents are not without toxicity and unwanted side effects. Recently Campath was reported to cause serious complications that prompted care providers to re-consider the use of Thymoglobulin, especially in renal transplant recipients[26-28].

Although short-term patient and graft outcomes have greatly improved, 5-year survival remains to be improved. In our series, 5-year survival was 40% for grafts and 60% for patients. While still better than the survival rates reported for recipients in the 2009 Transplant Registry report, there is still a long way to go to improve overall survival.

In summary, the overall short-term outcome for SBTx recipients has greatly improved since our first report. Changes in immunosuppressant protocol with introduction of induction agents and refinement of surgical techniques may play a role in this improvement. However, this improvement has yet to be reflected in long-term patient and graft outcomes and scientists and clinicians have many challenges to overcome.

COMMENTS

Background

Intestinal transplantation was made possible by the advancement in immunosuppressant medications (tacrolimus) and it soon became the treatment of choice for patients with irreversible intestinal failure. However, long-term patient and graft health and survival are challenged by the high incidence of rejection and sepsis. As a result, new methods of controlling the immune response in small bowel transplant (SBTx) recipients continue to emerge, such as the use of irradiated grafts to control the intestinal lymphatic tissues and the application of immune induction agent to control the immune system. None of these methods are without risks or side-effects; some of these complications are documented in this study.

Research frontiers

Although SBTx is a well-established procedure for patients with short gut syndrome, it is only in its infancy; researchers and clinicians are still looking for answers and solutions to the problems associated with this procedure. Reporting the outcomes from a well-known transplant center and documentation of the impact of the immune induction agents on the perioperative course constitutes valuable information for care providers.

Innovations and breakthroughs

The use of different immune modulation agents may impact short-term patient and graft outcomes, but not long-term outcomes. Zenapax proved to be the most notorious agent in causing more unwanted side effects and can significantly impact the intraoperative and hospital course. Campath became the most commonly used agent due to lower incidence of complications when compared to Zenapex. Recently, the use of Thymoglobulin has been rising after

reports of serious complications related to Campath, especially in renal transplant recipients. Tailoring an immunosuppressant regimen that is appropriate for a particular patient will be the optimal way to control and modulate the immune response without added risk of sepsis.

Applications

This study showed that SBTx recipients have better short-term outcomes with a theoretical advantage in that the graft can be removed in cases of graft-related problems without serious impact on the recipient's well-being. The results showed an improved patient 1-year survival (95%) when compared to series from other centers, which reflects the experience of the surgical team and/or improvement in the immunosuppression protocol and patient selection.

Terminology

This study defined post reperfusion syndrome according to the standard that is accepted by the anesthesiology team at our institution, which is not widely used outside our practice. The authors defined acute kidney injury (AKI) using the definition of the modified RIFLE (risk, injury, failure, loss, end-stage renal disease) criteria, as recommended by the Kidney Disease Improving Global Outcomes AKI guideline but without urine output data. This definition uses a 50% increase in SCr from the baseline (pre-operative value) or a 0.3 mg/dL increase within 48 h.

Peer review

The nature of a retrospective study may lead to important limitations on the identification and analysis of different confounding factors. However, the data we used in this study were carefully collected, maintained, and tabulated for each SBTx recipient as a part of our institutional policy, which gives credibility to the authors' study findings.

REFERENCES

1 **Abu-Elmagd K**, Reyes J, Bond G, Mazariegos G, Wu T, Murase N, Sindhi R, Martin D, Colangelo J, Zak M, Janson D, Ezzelarab M, Dvorchik I, Parizhskaya M, Deutsch M, Demetris A, Fung J, Starzl TE. Clinical intestinal transplantation: a decade of experience at a single center. *Ann Surg* 2001; **234**: 404-416; discussion 416-417 [PMID: 11524593]

2 **Planinsic RM**. Anesthetic management for small bowel transplantation. *Anesthesiol Clin North America* 2004; **22**: 675-685 [PMID: 15541930 DOI: 10.1016/j.atc.2004.06.007]

3 **Hilmi I**, Horton CN, Planinsic RM, Sakai T, Nicolau-Raducu R, Damian D, Gligor S, Marcos A. The impact of postreperfusion syndrome on short-term patient and liver allograft outcome in patients undergoing orthotopic liver transplantation. *Liver Transpl* 2008; **14**: 504-508 [PMID: 18383079 DOI: 10.1002/lt.21381]

4 **Calandra T**, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit Care Med* 2005; **33**: 1538-1548 [PMID: 16003060]

5 **Mehta RL**, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; **11**: R31 [PMID: 17331245 DOI: 10.1186/cc5713]

6 **Wong F**, Nadim MK, Kellum JA, Salerno F, Bellomo R, Gerbes A, Angeli P, Moreau R, Davenport A, Jalan R, Ronco C, Genyk Y, Arroyo V. Working Party proposal for a revised classification system of renal dysfunction in patients with cirrhosis. *Gut* 2011; **60**: 702-709 [PMID: 21325171 DOI: 10.1136/gut.2010.236133]

7 **Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group**. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Inter* 2012; **2**: 1-138

8 **Matsusaki T**, Sakai T, Boucek CD, Abu-Elmagd K, Martin LM, Amesur N, Thaete FL, Hilmi IA, Planinsic RM, Aggarwal S. Central venous thrombosis and perioperative vascular access in adult intestinal transplantation. *Br J Anaesth* 2012; **108**: 776-783 [PMID: 22362673 DOI: 10.1093/bja/aes016]

9 **Seisdedos Elcuaz R**, Conde García MC, Castellanos Monedero JJ, García-Manzanares Vázquez-de Agredos A, Valenzuela Gámez JC, Fraga Fuentes MD. [Central venous catheters-related infections in patients with parenteral nutrition]. *Nutr Hosp* 2012; **27**: 775-780 [PMID: 23114943 DOI: 10.3305/nh.2012.27.3.5729]

10 **Shaffer BC**, Modric M, Stetler-Stevenson M, Arthur DC, Steinberg SM, Liewehr DJ, Fowler DH, Gale RP, Bishop MR, Pavletic SZ. Rapid complete donor lymphoid chimerism and graft-versus-leukemia effect are important in early control of chronic lymphocytic leukemia. *Exp Hematol* 2013; **41**: 772-778 [PMID: 23689118 DOI: 10.1016/j.exphem.2013.04.015]

11 **Wu G**, Selvaggi G, Nishida S, Moon J, Island E, Ruiz P, Tzakis AG. Graft-versus-host disease after intestinal and multivisceral transplantation. *Transplantation* 2011; **91**: 219-224 [PMID: 21076376 DOI: 10.1097/TP.0b013e3181ff86ec]

12 **Mazariegos GV**, Abu-Elmagd K, Jaffe R, Bond G, Sindhi R, Martin L, Macedo C, Peters J, Girnita A, Reyes J. Graft versus host disease in intestinal transplantation. *Am J Transplant* 2004; **4**: 1459-1465 [PMID: 15307833 DOI: 10.1111/j.1600-6143.2004.00524.x]

13 **Akhter K**, Timpone J, Matsumoto C, Fishbein T, Kaufman S, Kumar P. Six-month incidence of bloodstream infections in intestinal transplant patients. *Transpl Infect Dis* 2012; **14**: 242-247 [PMID: 22093913 DOI: 10.1111/j.1399-3062.2011.00683.x]

14 **Reyes J**, Green M, Bueno J, Jabbour N, Nalesnik M, Yunis E, Kocoshis S, Kauffman M, Todo S, Starzl TE. Epstein Barr virus associated posttransplant lymphoproliferative disease after intestinal transplantation. *Transplant Proc* 1996; **28**: 2768-2769 [PMID: 8908049]

15 **Przepiorka D**, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825-828 [PMID: 7581076]

16 **Middleton SJ**, Pollard S, Friend PJ, Watson C, Calne RY, Davies M, Cameron EA, Gimson AE, Bradley JA, Shaffer J, Jamieson NV. Adult small intestinal transplantation in England and Wales. *Br J Surg* 2003; **90**: 723-727 [PMID: 12808622 DOI: 10.1002/bjs.4095]

17 **Todo S**, Reyes J, Furukawa H, Abu-Elmagd K, Lee RG, Tzakis A, Rao AS, Starzl TE. Outcome analysis of 71 clinical intestinal transplantations. *Ann Surg* 1995; **222**: 270-280; discussion 280-282 [PMID: 7677458]

18 **Kato T**, Tzakis AG, Selvaggi G, Gaynor JJ, David AI, Bussotti A, Moon JI, Ueno T, DeFaria W, Santiago S, Levi DM, Nishida S, Velasco ML, McLaughlin G, Hernandez E, Thompson JF, Cantwell P, Holliday N, Livingstone AS, Ruiz P. Intestinal and multivisceral transplantation in children. *Ann Surg* 2006; **243**: 756-764; discussion 764-766 [PMID: 16772779]

19 **O'Keefe SJ**. Candidacy for intestinal transplantation. *Am J Gastroenterol* 2006; **101**: 1644-1646 [PMID: 16863572 DOI: 10.1111/j.1572-0241.2006.00707.x]

20 **Pironi L**, Joly F, Forbes A, Colomb V, Lyszkowska M, Baxter J, Gabe S, Hébuterne X, Gambarara M, Gottrand F, Cuerda C, Thul P, Messing B, Goulet O, Staun M, Van Gossum A. Long-term follow-up of patients on home parenteral nutrition in Europe: implications for intestinal transplantation. *Gut* 2011; **60**: 17-25 [PMID: 21068130 DOI: 10.1136/gut.2010.223255]

21 **Trevizol AP**, David AI, Dias ER, Mantovani D, Pécora R, D' Albuquerque LA. Intestinal and multivisceral transplantation immunosuppression protocols--literature review. *Transplant Proc* 2012; **44**: 2445-2448 [PMID: 23026616 DOI: 10.1016/j.transproceed.2012.07.016]

22 **O'Keefe SJ**, El Hajj II, Wu T, Martin D, Mohammed K, Abu-Elmagd K. Endoscopic evaluation of small intestine transplant grafts. *Transplantation* 2012; **94**: 757-762 [PMID: 22955230 DOI: 10.1097/TP.0b013e31825f4410]

23 **Remotti H**, Subramanian S, Martinez M, Kato T, Magid MS. Small-bowel allograft biopsies in the management of small-intestinal and multivisceral transplant recipients: histopathologic review and clinical correlations. *Arch Pathol Lab Med* 2012; **136**: 761-771 [PMID: 22742549 DOI: 10.5858/

arpa.2011.0596-RA].]
24 **Giraldo M**, Martin D, Colangelo J, Bueno J, Reyes J, Fung JJ, Starzl TE, Abu-Elmagd K. Intestinal transplantation for patients with short gut syndrome and hypercoagulable states. *Transplant Proc* 2000; **32**: 1223-1224 [PMID: 10995920]
25 **Suzuki M**, Mujtaba MA, Sharfuddin AA, Yaqub MS, Mishler DP, Faiz S, Vianna RM, Mangus RS, Tector JA, Taber TE. Risk factors for native kidney dysfunction in patients with abdominal multivisceral/small bowel transplantation. *Clin Transplant* 2012; **26**: E351-E358 [PMID: 22694120 DOI: 10.1111/j.1399-0012.2012.01672.x]
26 **Adams PS**, Shapiro R, Hilmi IA. Postoperative cardiac tamponade after kidney transplantation: a possible consequence of alemtuzumab-induced cytokine release syndrome. *Transplantation* 2013; **95**: e18-e19 [PMID: 23380870 DOI: 10.1097/TP.0b013e31827d9757]
27 **Castelli R**, Gritti G, Cannavò A, Moreo G, Conti G, Reda G, Cortelezzi A. Successful management with intravenous immunoglobulins in alemtuzumab-induced acute inflammatory demyelinating neuropathy: clinical report of three patients. *Immunopharmacol Immunotoxicol* 2012; **34**: 717-720 [PMID: 22208918 DOI: 10.3109/08923973.2011.644295]
28 **Muzaffar M**, Taj A, Ratnam S. Aggressive posttransplant lymphoproliferative disease in a renal transplant patient treated with alemtuzumab. *Am J Ther* 2010; **17**: e230-e233 [PMID: 19918163 DOI: 10.1097/MJT.0b013e3181c08042]

P- Reviewer: Hardinger KL **S- Editor**: Zhai HH **L- Editor**: A
E- Editor: Zheng XM

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
www.wjgnet.com

World J Transplant 2013 December 24; 3(4): I-V
ISSN 2220-3230 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Transplantation (*World J Transplant*, *WJT*, online ISSN 2220-3230, DOI: 10.5500) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aim and scope

WJT covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, and xenotransplantation. The current columns of *WJT* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

We encourage authors to submit their manuscripts to *WJT*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

WJT is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 43 OA clinical medical journals, including 42 in English, has a total of 15471 editorial board members or peer reviewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJT* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in transplantation; (12) Brief Articles: To briefly report the novel and innovative findings in transplantation; (13) Meta-Analysis: Covers the systematic review, mixedtreatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, *e.g.*, the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJT*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of transplantation; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Transplantation

ISSN

ISSN 2220-3230 (online)

Launch date

December 24, 2011

Frequency

Quarterly

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/2220-3230/g_info_20100722180909.htm.

Indexed and Abstracted in
PubMed Central, PubMed, and Digital Object Identifier.

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJT* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified.

A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-3230/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to bpgoffice@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, *e.g.*, Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, *e.g.*, Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P <$

0.05, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, *e.g.*, PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-3230/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-3230/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2220-3230/g_info_20100725073445.htm.

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

Statement about anonymous publication of the peer reviewers' comments

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJT is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. All invited articles are published free of charge.

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com